USE OF FLUORESCEIN DYE FOR PERIOPERATIVE FLAP VIABILITY

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(MAY,2021)

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Dedicated

to

my family

&

Teachers

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Dr. Tushar Patial

ABBREVIATIONS

99m:	99 metastable
ALT:	Anterolateral thigh
BCC:	Basal Cell Carcinoma
CA:	Carcinoma
dL:	Decilitre
g:	gram
ICG:	Indo Cyanine Green
Kg:	Kilogram
Kr:	Krypton
LD:	Latissimus dorsi
Mg:	Milligram
MHz:	Megahertz
Mm:	Millimetre
Nm:	Nanometre
pH:	Potential hydrogen
PMMC:	Pectoralis major myocutaneous
PTFE:	Polytetrafluoroethylene
SSI:	Surgical site infection
UV:	Ultraviolet
Xe:	Xenon

Na:	Sodium
Tc:	Technetium
$T^{1/2}$:	half life
^{o}C:	Centigrade

ABSTRACT

Background: A successful flap is dependent on its vascularity. During dissection, flap viability may become compromised due to inadequate perfusion from the vascular pedicle(s) on which the flap is based. Various methods have been proposed to determine viability of a flap both, intraoperatively and postoperatively. The ideal method must be simple, accurate, reproducible and safe. The purpose of this study was to study the role of fluorescein dye for prediction of flap viability.

Materials & Methods: Thirty patients undergoing flap coverage for various defects were evaluated. Flaps were elevated and evaluated clinically for viability. Intravenous fluorescein was injected into the patients prior to flap inset and its course through the flap was monitored under ultraviolet light. Fluorescent areas were marked intraoperatively (size and location documented). Areas of viable flaps were documented on post-operative day 5 and comparisons made.

Result: The area of viable flap as determined using intra operative fluorescein dye administration corresponded to surviving flap area on post-operative day 5, in 27 of the 30 cases studied, giving positive predictive value of 90%. Overall, the dye had an accuracy of 99.03% compared to intraoperative clinical flap assessment accuracy of 95.70%. Marginal necrosis was seen in 16.6 % of all cases. No allergic reaction to the dye was observed.

Conclusion: - Accurate assessment of flap viability is an area of constant research. Use of intraoperative fluorescein dye for prediction of flap viability has an accuracy of 99.03% compared to intraoperative clinical flap assessment accuracy of 95.70%. In a few cases, the dye is prone to underpredict the area of viable flap tissue. The dye can be used to corroborate clinical asessment of viable flap tissue when used intraoperatively. The dye was found to be safe and easy to use.

Keywords: - Fluorescein dye, flap viability, marginal necrosis.

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INTRODUCTION

Flap reconstruction is used for coverage of defects throughout the various parts of the body. One of the major complications of flap surgery remains flap necrosis. When a flap is elevated, blood flow to the elevated segment of profoundly affected. Any disruption if flow can lead to a drop in perfusion pressure. Once this perfusion pressure drops below a critical closing pressure, distal flow to the tissue stops. This ultimately leads to flap necrosis unless flow can be established in the distal part of the flap.[1] Flap necrosis has been described in workhorse flaps like Pectoralis major in the range of 21 to 27% and in the range of 13 to 57% for the Trapezius flap.[2-5] Identification of adequate vascularity is paramount to prevent this complication. When a suspicion of flap compromise is suspected, immediate interventions can help salvage the flap and/or revise the surgical plan to undertake some other form of reconstruction. Successful salvage rates range from 28 to 90%, and rapid re-exploration is crucial to maximise the chances of flap salvage. If salvage is not possible or unsuccessful, other non-surgical methods can be employed with the possibility of some success.[6]

Surgeons rely on physical examination to assess the vascularity of flaps by measuring temperature, colour, capillary refill, turgor and pin prick. Several adjunctive measures have been proposed to aid the surgeon in his/her quest. These include the use of Doppler based instruments, tissue pH monitoring, skin temperature probes, tissue oxygen tension, near infrared spectroscopy, photoplethysmography, micro dialysis, flap glucose assessment as well as the use of fluorescent dyes amongst many others.[7] In some cases, areas with poor arterial flow are obvious. For example, presence of dusky colour, absent turgor, capillary refill, and a lack of dermal bleeding. However, areas of borderline perfusion are much more difficult to assess. Clinically, the skin colour may be pale, while turgor, capillary refill, and dermal bleeding may be present but reduced. In such patients, accurate prediction of the ultimate fate of the threatened skin may be

difficult even in the hands of an experienced surgeon, and the final outcome may not be certain for 2 to 3 weeks.[8] The use of dyes to delineate vascular anatomy has been well studied.[9-13] In our study of 30 patients, we proposed the use of Fluorescein dye to predict the viability of various flaps.

Fluorescein was first used by Ehlrich in 1882 and put into clinical use in 1931.[14] Fluorescein is a small water soluble molecule that rapidly diffuses out of capillaries and into extravascular tissue. The dye diffuses across the capillary fluid compartment within 10-15 minutes. It can then be measured as the intensity of fluorescence using UV light at the site of interest. The medical use of Fluorescein was popularized by Lange and Boyd in 1942.[15] Thereafter, Dingwall and Lord, used the dye to predict the viability of tubed flaps in 1943.[16] Fluorescein has been used in ophthalmology for corneal erosions and for fluorescence angiography. It has been used in experimental dermatology and it can be used as an intradermal injection, intravenous injection or topically.[17]

Fluorescein is hygroscopic orange red powder, which dissolves readily in water. When viewed under UV light, it assumes a yellow green colour. In our study, the predominant areas of observation were the skin and the muscle. Skin containing fluorescein appears yellow green when viewed under UV light. Although in darker individuals the presence of melanin makes visualization difficult. This can be remedied by de-epithelialization of the skin.[18] Muscle fluorescence is similar to skin but is less intense.[19] Fluorescence is a form of luminescence in which light energy is absorbed without dissipation. The test consists of injecting Fluorescein intravenously in a dose of 15 to 30 mg/kg. If the vascularity of the flap is maintained, fluorescence is visible in the flap till the distal end thereby confirming the viability of the flap. If fluorescence is not present, further interventions to salvage or abandon the flap can be made expeditiously. Numerous methods have been used to evaluate fluorescence. These include the use of Wood's lamp, Fluorometer, use of a camera with interference filter and use of camera with plexiglass filter. Despite the use of newer technology, traditional

measurement has proven to be as accurate as any of the newer methods.[20]

This method of assessment of flap viability is simple, cost effective and requires little special equipment. It is more objective than traditional methods of flap monitoring such as observing colour, turgor or pin prick examination. Not only does such a method provide adequacy of flap perfusion, but it also allows for an objective comparison with respect to traditional methods of assessment of flap viability.

AIMS AND OBJECTIVES

1. To validate/negate the use of Fluorescein dye for flap viability in Plastic surgery.
2. To compare the use of Fluorescein dye to clinical examination of flap for viability.

REVIEW OF LITERATURE

Despite rapid development in the field of reconstructive surgery, one core issue that persists to this day is the inability to accurately predict the viability of a flap.

Although many attempts have been made to elucidate elaborate as well as simple adjuncts to aid the surgeon in his decision making, the ideal technique remains to be found. Most techniques remain imperfect or expensive.[21]

Creech and Miller proposed that the ideal technique for assessment of flap viability should fulfil the following tenets:[7]

1) Simple and harmless to the patient
2) Rapid, repeatable, reliable, recordable and rapidly responsive
3) Accurate and inexpensive
4) Objective and applicable to all kind of flaps
5) Equipped with a simple display that could alert relatively inexperienced personnel to the development of circulatory impairment.

The most common technique used to check for flap viability is “Clinical examination”. This may include all or a combination of the following:[22]

1) Colour
2) Turgor
3) Temperature
4) Capillary refill time
5) Bleeding following pin prick.

Although these methods are cheap and repeatable, they have certain limitations. The appreciation of colour, for example, is dependent on ambient lighting conditions.[23] Hence, these techniques have to be supplanted by other more objective and reliable methods.

Methods of flap monitoring

Clinical tests

Of all the techniques available, clinical assessment is the most commonly used.[24] Clinical assessment generally consists of some combination of the following:

1.Skin colour
2. Skin turgor
3. Surface temperature
4. Bleeding alter pinprick
5. Capillary refill time

These bedside tests are cheap, repeatable and easy to perform. In spite of technological advances, clinical examination is still deemed to be the “gold standard” for post-operative flap monitoring.[7] However, there are certain limitations that these tests have. The clinical examination is dependent upon a portion of flap remaining visible and assessment is difficult if not impossible to perform when the entire flap is covered by native skin and invisible from the outside. Since, the entire flap is not being examined at a time, this method may be subject to sampling error.[18]

If the healthcare provider cannot visualize a buried flap, she or he must rely on adjunctive methods in order to properly monitor the flap. Colour changes vary when the occlusion occurs within the arterial or the venous system. When arterial occlusion takes place, the flap takes on a pale look, but becomes cyanotic with purple colouration when venous occlusion takes place.[25] However, colour perception is influenced by ambient lighting, and the colour of the transferred tissue may be different from that of the recipient site.[23] Temperature may be assessed by touch, temperature probes, temperature-sensitive tape, or a hand-held noncontact thermometer. A drop in temperature alone may suggest vascular occlusion but is often used in combination with other findings to establish a diagnosis.[25] Surface temperature is dependent on many factors including blood flow and changing metabolic rate of the underlying muscle.[26] Assessment of the skin temperature may be unreliable and dependent on ambient conditions at the time.[26] Capillary refill changes can be observed with arterial and venous occlusion. With arterial occlusion there is usually sluggish to absent capillary refill. In the setting of venous occlusion, the capillary refill is still present and is usually brisk (< 1 second). Capillary refill time too has a wide variation amongst its users due to little standardization.[27]

Changes in size and turgor are evident during arterial and venous occlusion. With arterial occlusion, the flap size and turgor are decreased. With venous congestion, the flap size and turgor are increased.[25] Skin turgor by itself has little proven diagnostic value and is dependent of the volume status of the patient.[28] Capillary bleeding on pinprick has been considered by some as a reliable indicator of microvascular circulation by some, while others suggest its significance is only valid at extremes of flap viability.[19,22] A scratch test is sometimes used to evaluate local blood flow. The flap may be scratched with an 18-gauge needle to assess blood flow. If arterial occlusion is present, the flap will exhibit slow or absent bleeding. If venous occlusion occurs, rapid bleeding of darker than anticipated blood is observed.[25] The effectiveness of clinical monitoring is high with success rates ranging from 85 to 95%.The majority of components of clinical assessment (including skin colour, temperature, capillary refill assessment) require no additional equipment, and the cost of needles for pinprick assessment and other equipment such as tongue depressors for the assessment of oral cavity flaps can be assumed to be negligible. . Clinical flap assessment, therefore, remains an inexpensive, risk-free, and relatively efficient method of postoperative free-flap monitoring.[29]

Indocyanine green

Indocyanine green (ICG) is a fluorescent dye that has been adapted for use in flap surgery. When ICG is exposed to wavelengths of 750 to 810 nm, it emits fluorescence that peaks at 840 nm. The light needed foe excitation is generated by a near infrared light source and the emission is captured by a digital camera equipped with a special filter.[30] It has high sensitivity (90.9%) and accuracy (98.6%) in predicting tissue transfer survival.[31] However, its limited depth detection (less than 1 cm) and relatively high cost of equipment needed are practical limitations reducing the dye to an adjunctive tool when used for flap assessment.[32]

Doppler examination

Most commonly used as an external hand-held probe with a frequency of 8 MHz, the device bounces high frequency sound waves off the underlying vasculature. In case of arterial occlusion, the signal changes from triphasic to a monophasic signal or complete absence. Venous occlusion may not produce any observable changes during the early stages, however in the late stages of venous occlusion, the arterial signal may have a "water – hammering" characteristic.[25] Despite a relatively high cost of the device, the probes are reusable and thus makes this an inexpensive and risk free method of flap monitoring.[29]

Implantable doppler

The implantable doppler probe was first introduced in 1988, and the advantage of such a device is that it allows for real time information and direct monitoring of blood flow.[33] A tiny 1 mm probe is attached to an expanded PTFE cuff that is placed around either the arterial or the venous anastomosis.[34] The device however has a false positive rate of 8 to 17 percent, which are often attributed to probe malfunction or malpositioning, leading to unnecessary reoperation.[35] Also, the device has an estimated cost of about $300 per probe, making it an expensive investment.[36]

Colour duplex ultrasonography

This technique uses ultrasound technology to visualize flow through the underlying vasculature. Similar to the handheld device, the device is non-invasive and can be useful in the assessment of flaps, especially those which are buried. The equipment has high cost, and requires a trained ultrasound technician, a radiologist as a surgeon to perform and interpret the examination. Due to high resource intensity, the use of this technique as the sole method of flap monitoring becomes difficult.[29]

Tissue pH

pH monitoring has been used in the assessment of flap monitoring. Impairment of tissue blood flow leads to an increase in the anaerobic metabolic products and an accumulation of acid metabolites. This increases the concentration of hydrogen ions leading to a drop in pH.[7]

Radioactive isotopes

The basis for the use of radioactive isotopes is the Fick's diffusion principle. The measurement of decay of radioisotopes is measured and an inference can be made regarding flap perfusion. Numerous isotopes have been used for assessing tissue clearance. These include ^{85}Kr, ^{133}Xe, ^{22}Na and ^{99m}Tc.[7]

Technetium – 99m

Technetium 99m Sestamibi scintigraphy has been described by multiple authors for use in determination of flap viability.[37-39] Its short half-life of 6 hours allows for two scintigraphic examinations within 24 hours.[40] The most useful application of the technique is in assessment of vascularized bone grafts. Radioactive waste contamination and unavoidable radiation to the patient are two major drawbacks that have limited the use of this technique more widely.[7]

Surface temperature monitoring

Comparative temperature difference between the surface of the flap and its surrounding skin can be used as an indicator for flap compromise. A temperature difference of greater than 3^oC between the flap and its adjacent skin is indicative of arterial compromise. A difference between 1^0C and 2^oC is suggestive of venous compromise. The disadvantage is of this technique is that it is unreliable for intraoral flaps and cannot be used in buried flaps.[41] Although easy to perform and repeatable, it has long been known that blood flow to a flap is related to blood pressure and microcirculation but not in a consistent manner.[42] The temperature readings are also affected by room temperature, convection and individual variations in body temperature.[43] Reliability of the technique has also been questioned because of its inability to detect changes before flap failure.[44]

Tissue oxygen tension

Measurement of oxygen tension is often used as a tool for monitoring revascularized digits, toe to hand transfers and replanted limbs.[45] The pulse oximeter is made up of two separate light emitting diodes. These function at 660 nm (visible red) and 940 nm (infrared). The difference

between the oxyhaemoglobin and reduced haemoglobin allows for measurement of oxygen saturation.

Photoplethysmography

A green light emitting diode is used to transmit light into a tissue. The light is reflected from haemoglobin in dermal capillary red cells and is received by a photo detector. This light is then analysed along a frequency spectrum. This allows for a method to distinguish between perfused and non-perfused tissue.[46]

Microdialysis

Microdialysis is a microsampling technique that allows for measurement of the biochemistry in specific organs. A double lumen microcatheter is introduced into the tissue. Perfusion fluid is slowly pumped across through the catheter with collection of the dialysate. This fluid is then analysed. Glucose, glycerol and lactate concentrations are measured in the flaps and compared to a standard reference. In cases of arterial thrombosis glucose levels tend to fall to unmeasurable levels, lactate levels rise for a period before a sudden fall and glycerol levels increase to a very high level.[47]

Near infrared spectroscopy

This technique employs the principle of optical spectrometry to measure haemoglobin content and oxygenation in local tissues. Selective light absorption by oxygen dependent tissues like haemoglobin yields a spectrum which allows for calculation of deoxygenated and oxygenated haemoglobin.[48] The ‘depth of penetration of near infrared light is said to be 20 mm.[49] The technique is able to identify early signs of arterial and venous thrombosis prior to any clinical signs of flap failure.[49,50] The device costs about $16500 for the monitoring device and $150 for the disposable sensor.[51]

Capillary lactate and glucose

Capillary glucose and lactate levels can predict vascular thrombosis.[52] Bashir et al. determined that at glucose levels below 62 mg/dL, the sensitivity for detecting flap compromise was 90%.[53] Serum lactate levels too have been used to measure flap viability. These levels show a gradual rise for about 8 hours before normalizing.[54]

Fluorescein

History

Sodium fluorescein was synthesized in 1852. Thirty years later Ehrlich used sodium fluorescein for the first time to study the secretion, flow and absorption of the aqueous humour in the rabbit's eye.[55] In 1922 Kock determined the circulation time by injecting sodium fluorescein into a vein of one arm and collecting samples of blood from a vein of the other arm and examining them for the first appearance of fluorescence.[56] Lange and Wollheim demonstrated that the dye could be seen in the blood vessels of the lip when observed appropriate light.[57] Lange and Boyd made an extensive study of capillary blood flow, and with the use of ultraviolet light they made the dye become visible.[58] This led to a series of clinical studies of capillary blood flow, circulation time and tissue viability in various clinical situations
in association with various surgical techniques and conditions, such as flaps, acute arterial embolism and thrombosis and vasospastic disorders. Lange and Krewer introduced the dermofluometer, which permitted objective measurement of fluorescence.[56] In clinical practice sodium fluorescein has been used to evaluate the viability of different tissues, for example the small intestine in strangulated hernia, tubed flaps, skin sloughs at the time of operation, colonic anastomosis and flaps during breast reconstruction.[9,10,16,59,60]

The use of sodium fluorescein has led to the development of several techniques for the study of the circulation: fluorescein angiography allows visualization of the retinal vessels and study of the cerebral circulation, since the dye does not leak from the circulation under normal conditions.[61] Increased permeability in pathological conditions causes leakage of the dye and can be measured quantitatively.[62]

Physical and biochemical characteristics

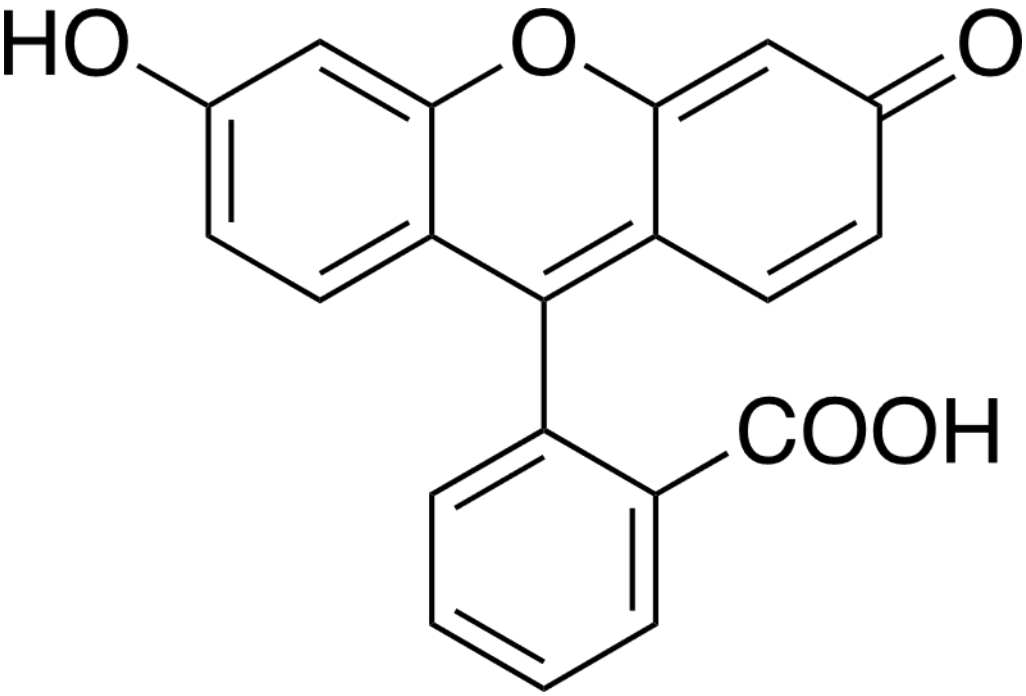

Figure 1: Structure of Fluorescein salt

Fluorescein is a resorcinolphthalein with a molecular weight of 376.27 Daltons and a radius of 0.55 nm. In clinical medicine it is used as a sodium salt. Sodium fluorescein is an orange-red, odourless, almost tasteless, hygroscopic, water-soluble powder. The aqueous solution is red in colour but presents a green fluorescence that is maximal when exposed to a light source with a wavelength 3600 to 4000 Angstroms, the ultraviolet range. Ultraviolet light at this spectrum briefly increases an electron to a higher energy level within the fluorescein molecule and releases a photon of light as it returns to its resting state. This photon leads to gold-green fluorescence at a wavelength of 5600 Angstroms. When diluted, the solution is yellow-green. The fluorescence disappears when the solution is acidified and reappears when it is alkalinized. Sodium fluorescein is soluble in one to ten parts of alcohol, and insoluble in chloroform and ether. A 3.34% solution in water is iso-osmotic with serum. A 0.5% solution has a pH value of 8.2 to 8.7.[56]

Metabolism

When injected intravenously, sodium fluorescein is free and dissociated and does not bind to proteins within the first two minutes.

Thereafter it binds successively to albumin but not to globulins or fibrinogen.[63] About 17% of the dye binds to the erythrocyte surface by adsorption.[64] In the microcirculatory system it diffuses through the capillaries to the interstitium and back to the circulation. Diffusion is limited, however, by the size of the capillary pores, which differs in different tissues. In the mucosa of the small intestine and in the muscularis layer, diffusion is unrestricted because of the large size of the capillary pores, which have a ten times greater diameter than sodium fluorescein.[65] Diffusion is partly restricted in the skeletal muscle, and in brain vessels diffusion does not occur on account of the absence of pores in the brain capillaries.[66] Elimination takes place through the liver and kidneys.[58] No metabolism occurs in these organs.

Dosage

Sodium fluorescein is given at a dose of 5-7 mg/kg body weight (range 5-15 mg/kg) as a 5% or 10% solution in studies of the circulation, both in skin and viscera.[67]

Toxicity

There are no reports of serious reactions after oral administration of sodium fluorescein, and as much as 6g has been given without any toxic effects.[58] After intravenous injection of fluorescein a very low incidence of serious adverse reactions and a high incidence of minor untoward effects have been reported. Fatal complications have been reported to occur at a rate of one case per 49,557 angiographies and one case per 222,000 angiographies.[68, 69] No deaths were observed in a prospective study of 2,789 angiographies.[70] The first anaphylactoid reaction to intravenous fluorescein was reported in a patient in 1968.[71]

Severe adverse reactions:

The overall frequency of such reactions was reported to be between one case per 18,020 angiographies to one case per 1,900 angiographies.[68,69] Tonic-clonic seizures occurred in 1:13,700, respiratory adverse reactions in 1:3,800 and cardiac problems in 1: 5,300 angiographies.[69] Kwiterovich et al. found two isolated cases of dyspnoea and two of syncope.[70]

Minor adverse reactions:

Nausea, vomiting, itching and flushing may occur at frequencies of 0.05 to 21 %. Yanuzzi et al. reported an estimated frequency of 1-10 % and Kwiterovich et al. a frequency of 4.8 %.[69,70] Watson et al., found an adverse reaction frequency of 48 % among individuals who had previously had an adverse reaction.[72] Turetta et al. noted a significant decrease in the plasma ionized calcium level in 84 patients during fluorescein angiography.[73] Extravascular administration of 10 ml of 5 % solution in the cubital area caused pain but no tissue damage.[13]

Fluorescein is a nontoxic dye which diffuses into the extracellular space.[17] When exposed to ultraviolet light, it gives off a yellow green fluorescence at a wavelength of 510 to 600 nm. This fluorescence persists in the extracellular space. Thus, the intensity of skin in living tissue is an expression of extracellular fluorescence rather than the amount of blood flow or intravascular concentration.[19] The dye appears as bright yellow while areas which have no perfusion appear as dark blue.[18] The fluorescence is seen predominantly from the blood vessels containing the dye in the superficial dermis.[74] The yellowish discolouration caused by the dye persists in the palm, the sole and the conjunctiva till about 24 hours. In urine, fluorescence persists till about 36 hours.[18]

Fluorescein was initially used by Ehrlich in 1882, and then introduced to clinical use in 1931.[17] Skin fluorometry after an intravenous injection was studied by Lange and Boyd, who used the method to evaluate microcirculation of the skin.[15] Dingwall and Lord used the dye to predict the viability of tubed flaps in 1943.[7]

In 1973, Thorvaldsson and Grabb, studied 19 patients who required skin flaps with Fluorescein dye. In their study they discovered that 5 patients who had non-fluorescing areas after administration of Fluorescein necrosed post operatively.[75]

In 1977, McCraw et al. suggested that despite its long-established record of use in various clinical scenarios, Fluorescein remains underused by reconstructive surgeons. The authors believed that the reasons for not using the dye were: -

1. The inherent safety of the dye is not well recognized.
2. The reliability of the test to predict flap viability was not well documented.
3. The limits of interpretation of the test not clearly defined.

In their study on 159 axial skin flaps and 126 myocutaneous flaps, 5% or 10% Fluorescein was injected via a venous catheter over a five-minute period with dosage as per body weight. The technique correctly predicted flap survival within a distance of 3 to 5 mm. They recommended routine fluorescein use whenever a major flap was manipulated. Of note is the fact that the authors had performed thirty cases before being convinced the dye is uniformly correct. The authors also described spotty fluorescence and its interpretation for flap viability. The authors also found that capillary refill and flap colour were only valid in a gross manner.[19]

In 1979, Dibbell et al. developed a Fluorescein based quantitative method for predicting viability of skin flaps. In their study they noted that a few areas of non-fluorescence also survived, suggesting that these areas survived as a graft rather than a flap. They noted that spotty fluorescence was always present beyond the area of bright fluorescence and signalled reduced viability. The non-fluorescent area was significantly less than the dead area.[13]

In 1980, Bardach and Panje, performed a study to evaluate the accuracy of the fluorescein test in predicting skin flap viability. They discovered that the surviving area was significantly greater than the staining area of the flap. They concluded that the fluorescein dye test should only be used for an approximate estimation, not for accurate prediction of flap survival.[76]

Myers at al. in 1985, compared eight methods to predict the viability of skin flaps using Fluorescein. Both low dose and high dose experiments were carried out using a fluorometer, camera with filters, and the traditional Wood's lamp. The authors discovered that the traditional Wood's lamp is just as accurate as any other method.[20]

Pang et al., in 1986 suggested that a visual Fluorescein test performed 18 hours after flap surgery provided an accurate prediction of actual

skin viability in flap surgery in pigs. However they noted that there is an underestimation of about 20% when given one hour after flap elevation.[77]

Denneny et al., in 1986, published an experimental study on rats using Fluorescein elimination to determine perfusion of island flaps. Through their experiments they determined that total occlusion of either the arterial or the venous supply of an island flap resulted in immediate cessation of Fluorescein dye elimination. Incomplete arterial obstruction also led to decrease in $t^{1/2}$ and washout of the dye. They concluded that use of Fluorescein dye offers an advantage in assessment of an island flap.[78]

Galla et al., in 1992, performed a comparative analysis of tissue fluorescence as related to capillary perfusion in random pattern flaps. Twenty-four hours after flap elevation, the region of interest was examined under a microscope. The rate of correctly predicted regions of interest to survive was compared to the specificity for tissue fluorescence. In these experiments, they concluded that skin flap fluorescence can be used for assessment of nutritive blood flow, viability of skin tissue and tissue survival in these flaps.[79]

Burnam in 1993, used 10% intravenous fluorescein to study the vascularity of the subcutaneous pedicled extension flap. Fluorescence was graded on a visual basis as “poor”, “fair”, “good” or “excellent”. They studied eleven cases for intraoperative vascularity using the dye and concluded that fluorescence at fifteen minutes was the best indicator of viability. In the study, it was also found that some flaps appeared dusky in the beginning, but fluorescence gradually improved over time, suggestive of decreasing vasoconstriction of the flap circulation.[80]

Issing et al. in 1996, performed an experimental study in rats to compare pedicled skin flap viability by pH, temperature and fluorescein. They concluded that the method which gave the most exact prediction about viability was the fluorescein staining of the flaps.[43]

Bhattacharya et al. in 2006, conducted an experimental as well as clinical study examining the fluorescein technique for per operative evaluation of vascularity. Thirty patients with flaps of various compositions were part of the study. 20% Fluorescein dye was given twenty minutes after dissecting the flaps. After this the flaps were examined under ultraviolet light. Yellow discolouration of then skin disappeared after 36 hours. No complications were observed and all flaps showed good vascularity without loss of flap. They suggested that the method was an easy, safe and reliable method to determine vascularity. The authors also pointed out that, since the entire flap can be visualized at one time, this method is also not prone to sampling error.[18]

In 2008, Losken et al. performed a study on fifty consecutive peri areolar mastectomy flaps, which were evaluated for skin flap viability using Fluorescein dye. After completion of mastectomy, skin flaps were examined for viability clinically and then Fluorescein dye was given. The patient was the examined after fifteen minutes under Wood's lamp. Areas of non-fluorescence were marked with a pen. Flap survival was documented at 1 to 2 weeks. In their study, the Fluorescein test had a positive predictive value of 96%. They concluded that the test is sensitive, but underpredicts viability. The authors noted that the dye can be helpful in clinical situations where perfusion seems marginal. They suggested that any area of non-fluorescence >4 cm^2 typically does not survive.[11]

In 2014, Lim et al. used fluorescein dye in degloving injuries. For 13 cases, with degloving injuries, intravenous fluorescein was used to determine the viability of avulsed skin flaps. Non – fluorescent areas were marked after fifteen minutes and these were covered with full thickness grafts. The authors concluded that fluorescein dye is a simple yet cost effective tool for evaluating flap viability.

In 2015, Beckler et al. performed a retrospective analysis for comparing skin paddle perfusion in those undergoing oromandibular reconstruction with fibular flap using clinical findings, indocyanine green and fluorescein dye. They noted that some areas which were at

risk as per the Fluorescein dye where not detected by Indocyanine green dye. These areas did not undergo necrosis. This suggested that the Fluorescein dye produces some false positive results. The authors further went on to suggest that the use of an intra operative dye may reduce the risk of partial necrosis in these patients.[8]

In 2018, Bongetta et al. reported on the use of Fluorescein dye for the evaluation of naso-septal flap perfusion. After applying a customized yellow filter to an endoscope, a dose of fluorescein equal to 5 mg/kg body weight is administered intravenously. After about 15 seconds, green staining is progressively visible in the perfused tissue. The technique was found to be accurate and cost effective.[81]

MATERIALS AND METHODS

SOURCE OF DATA

A prospective study was conducted on 30 patients operated for various defects requiring flap coverage by the Department of Plastic surgery, Dayanand medical college and hospital, Ludhiana. Those part of the study adequately satisfied inclusion criteria and none of the exclusion criteria.

METHOD OF COLLECTION OF DATA

Inclusion criteria

The study was conducted on all consenting patients who underwent any type of flap surgery beginning July 1, 2019 till completion of study.

Exclusion criteria

1. Patients who failed mandatory intradermal hypersensitivity test pre-operatively.
2. Patients who had a history of hypersensitivity to Fluorescein.
3. Patients who were breast feeding.

Procedural details

Pre operatively, all patients underwent an intradermal test dose of 0.05 mL. This was evaluated 30-60 minutes after injection to check for hypersensitivity.

For free flaps, the dye was injected after completion of the arterio-venous anastomosis. The flap was then examined under ultraviolet light using a Wood's lamp. For all other flaps, the dye was injected intravenously after dissecting the flaps.

For fascio-cutaneous flaps, an area of 2x2 cm^2 was deepithelialized to allow for visualization of Fluorescein. After a few minutes, the operating room lights were turned off and the flap was examined under ultraviolet light using a Wood's lamp (as pictured below).

Figure 2: Wood's lamp for observing fluorescence under ultraviolet light

Areas of impaired perfusion (if any) were examined, marked and documented.

Clinical examination included colour, capillary refill and needle prick examination at the time of surgery. The areas of adequate viability and/or non-viability were marked and documented onto a graph paper detailing the dimensions of the flap.

Outcome

Outcome analysis was performed to compare clinical assessment of flap viability versus fluorescein dye guided assessment by the plastic surgery resident on post-operative day 5. Photo documentation was performed, and the areas of necrosis/ non-viability were documented onto a graph paper detailing the areas of viable tissue and compared with operative assessment.

Duration of study

From July 1, 2019 till completion of study

STATISTICAL ANALYSIS

Data was described in terms of range; mean ± standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups was done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test was performed and exact test was used when the expected frequency was less than 5. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

OBSERVATIONS AND RESULTS

1. Age

In this study, the maximum number of patients were of the age group less than 30 years of age. The average age in our study was 39.43 years, with the age range between 20 and 71 years.

Table 1: Age

Age	No. of cases	Percentage
< 30	12	40.0%
31-40	7	23.3%
41-50	6	20.0%
> 50	5	16.7%
Total	30	100.0%

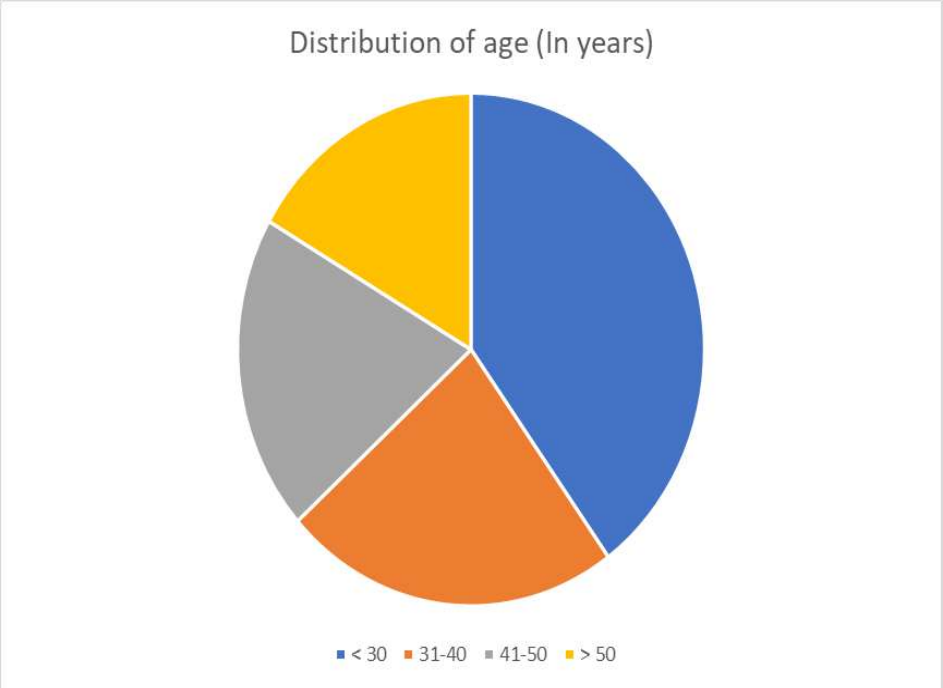

Figure 3: Age

2. Sex

In this study, 22 cases (73.3%) were males and 8 cases (26.7 %) were females.

Table 2: Sex

Sex	No. of cases	Percentage
Females	8	26.7%
Males	22	73.3%
Total	30	100.0%

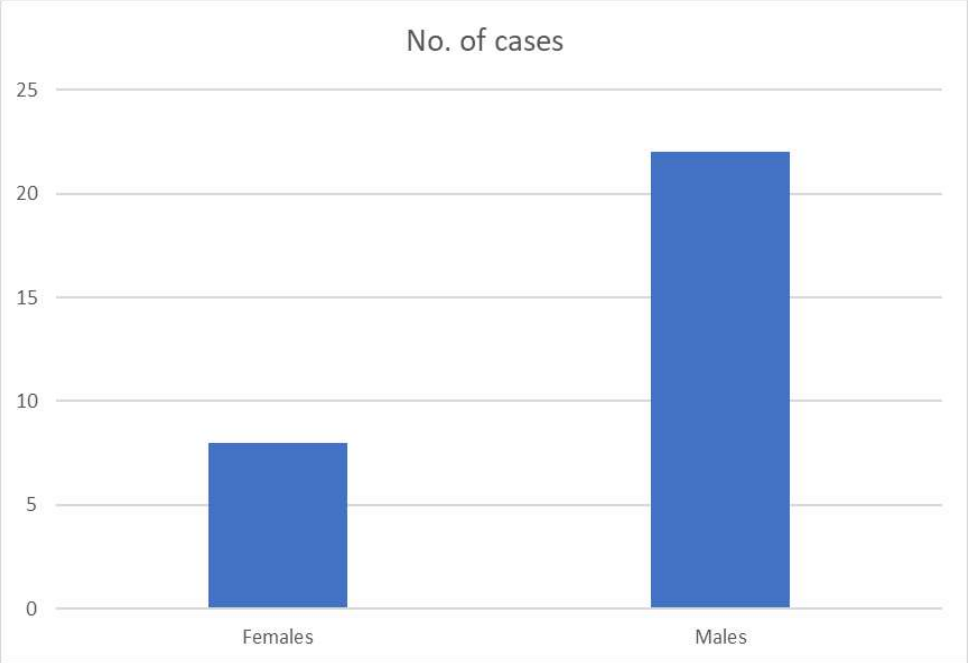

Figure 4: Sex

3. Body weight

In this study, we divided the body weight of the study sample into three arbitrary groups; patients weighing less than 50 kilograms, patients between 51 and 60 kilograms and patients weighing more than 60 kilograms of weight. This weight was taken at the time of admission.

The largest group of these, consisting of 13 patients, weighed between 51 and 60 kilograms accounting for 43.3 % of all cases. The smallest group, consisting of 8 cases, weighed above 60 kilograms. The average body weight was 56.43 kilograms with the weight range between 32 and 90 kilograms.

Table 3: Body weight classes

Body Weight (Kilogram)	No. of cases	Percentage
< 50	9	30.0%
51-60	13	43.3%
>60	8	26.7%
Total	30	100.0%

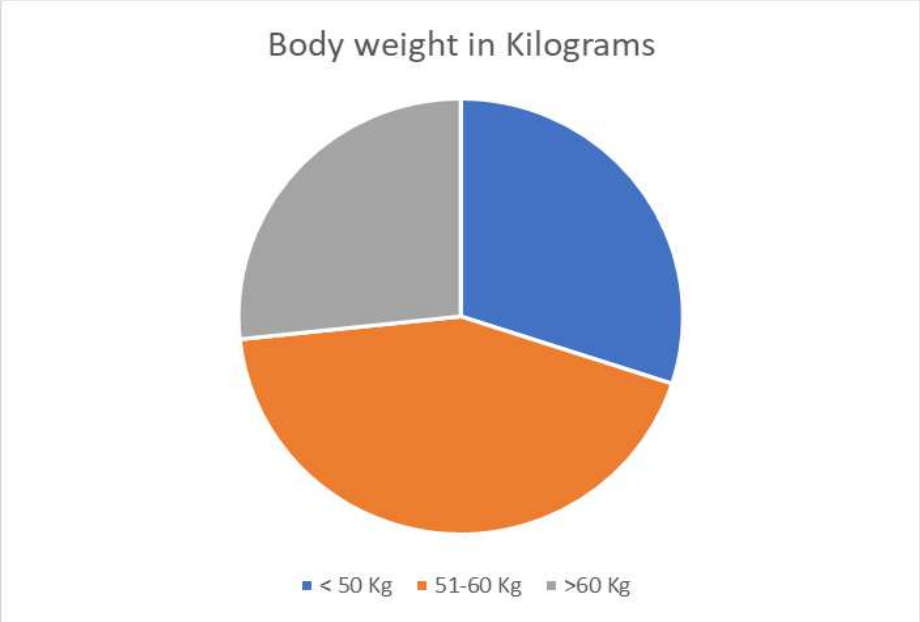

Figure 5: Body weight

4. Etiology of defects

In this study, the most common etiology of defects was cancer of the buccal mucosa accounting for 6 cases (20%) of all cases. Other etiologies are elaborated in the table below.

Table 4: Etiology of defects

Etiology of defect	No. of cases	Percentage
BCC LEFT CHEEK	1	3.3%
CA LOWER LIP	1	3.3%
ANTEROLATERAL THIGH DEFECT	1	3.3%
CA BUCCAL MUCOSA	6	20.0%
POST TRAUMATIC HEEL DEFECT	2	6.7%
POST TRAUMATIC LOWER 1/3RD LEG DEFECT	3	10.0%
POST BURN CONTRACTURE UPPER LIMB	1	3.3%
POST BURN RAW AREA DORSUM HAND	1	3.3%
POST ELECTRIC BURN RIGHT FOREFOOT DEFECT	2	6.7%
POST ELECTRIC BURN SCALP DEFECT	1	3.3%
POST TRAUMATIC RIGHT DORSUM HAND DEFECT	1	3.3%
RIGHT HAND DEGLOVING INJURY	2	6.7%
POST TRAUMATIC MIDDLE 1/3RD LEG DEFECT	3	10.0%
POST TRAUMATIC UPPER 1/3RD LEG DEFECT	2	6.7%
POST TRAUMATIC VOLAR FOREARM DEFECT	1	3.3%
POST TRAUMATIC DEFECT LEFT MASTOID	1	3.3%
STAGE 4 SACRAL PRESSURE SORE	1	3.3%

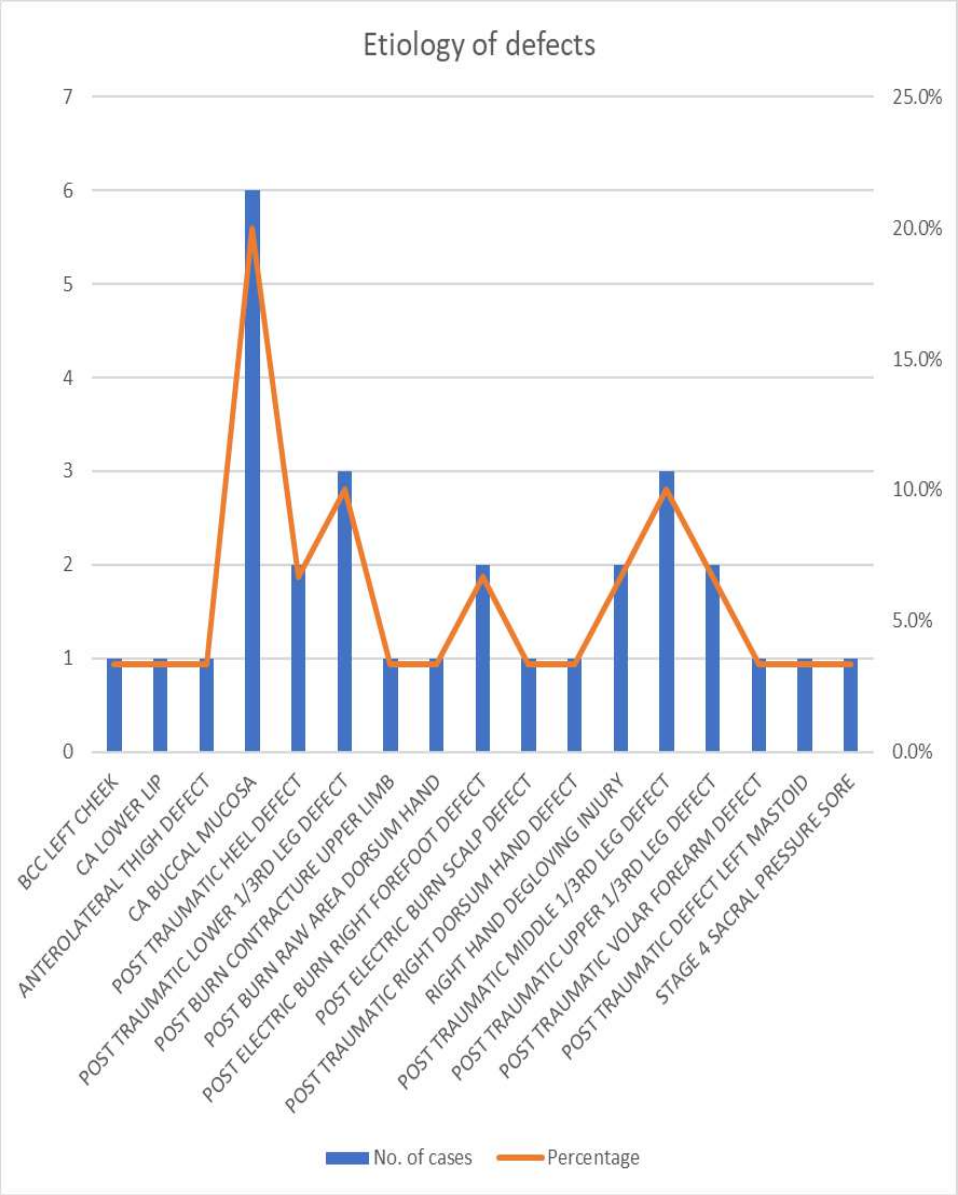

Figure 6: Etiology of defects

5. **Anatomical distribution of defects.**

In this study, the most common site of the defect was the lower limb accounting for 14 cases (46.6%) followed by head and neck defects accounting for 9 cases (30 %). Other sites being the upper limb in 6 cases (20%), and back in 1 case (3.3%).

Table 5: Anatomical distribution

Area of defect	No. of cases	Percentage
Upper limb	6	20 %
Lower limb	14	46.6 %
Head and neck	9	30 %
Back	1	3.33 %

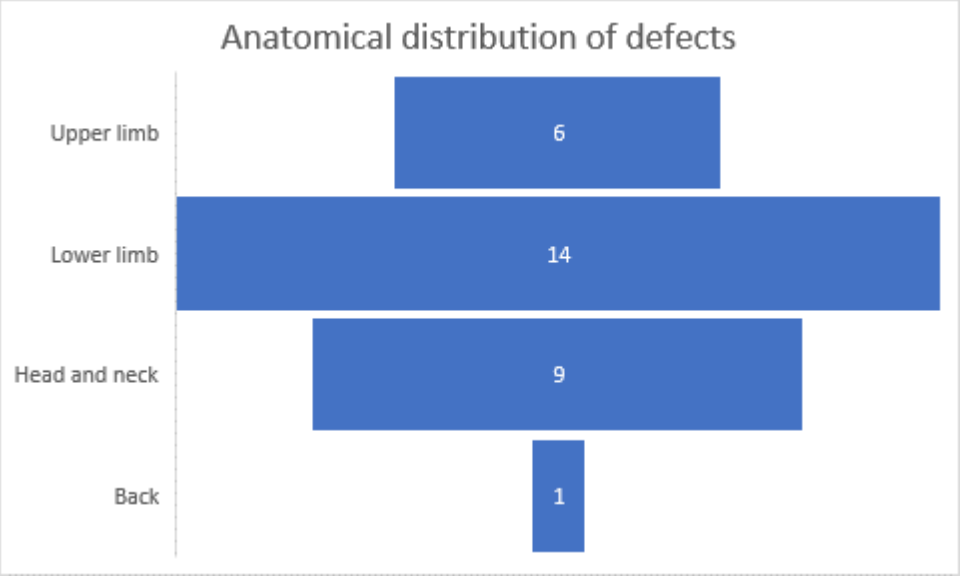

Figure 7: Anatomical Distribution

6. Type of flap

In this study, the groin flap was the most common type of flap performed accounting for 5 cases (16.7 %), the cross-leg flap and the transposition flap were the second most common type of flap done accounting for 4 cases each (13.3 %). The other flaps part of this study are stated as below.

Table 6: Types of flap

Type of flap	No. of cases	Percentage
ALT FASCIOCUTANEOUS FREE FLAP	2	6.7%
CROSS LEG FLAP	4	13.3%
FASCIOCUTANEOUS ROTATIONAL ADVANCEMENT FLAP	1	3.3%
LD FREE FLAP	1	3.3%
PMMC FLAP	3	10.0%
RADIAL ARTERY FREE FLAP	3	10.0%
MUSTARDE ADVANCEMENT FLAP	1	3.3%
PEDICLED LD FLAP	1	3.3%
REVERSE SURAL ARTERY FLAP	2	6.7%
GROIN FLAP	5	16.7%
GASTROCNEMIUS FLAP	2	6.7%
SPLIT ABDOMINAL FLAP	1	3.3%
TRANSPOSITION FLAP	4	13.3%

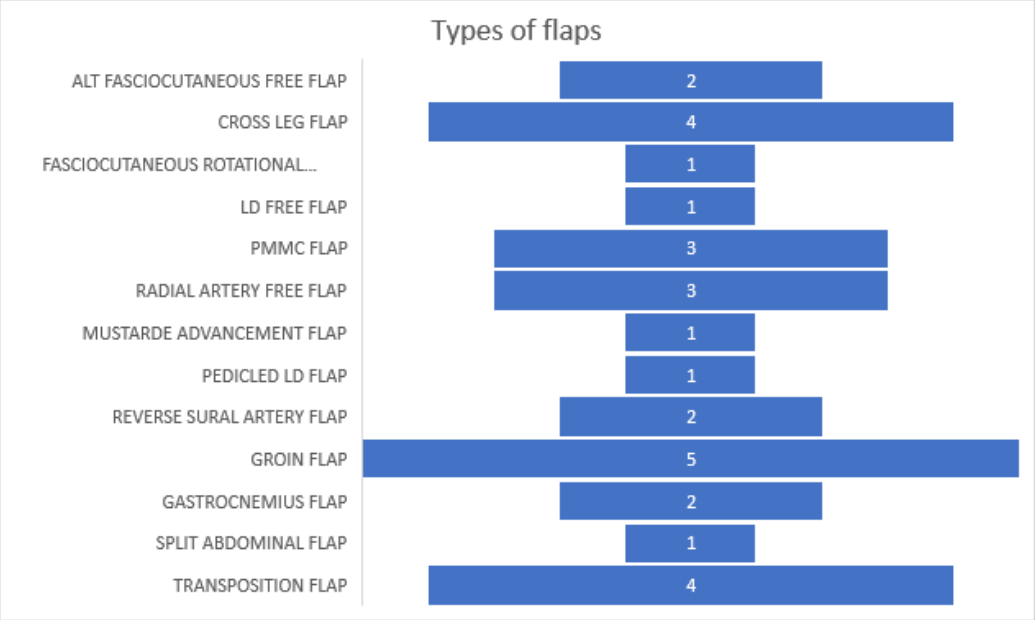

Figure 8: Types of flap

7. Distribution of flaps

In this study, out of 30 flaps, 16 flaps were distant flaps (53.3 %), 8 flaps were regional flaps (26.6%) and 6 flaps (20%) were local flaps.

Table 7: Distribution of flap

Type of flap	No. of cases	Percentage
Local flaps	6	20 %
Regional flaps	8	26.6 %
Distant flaps	16	53.3 %

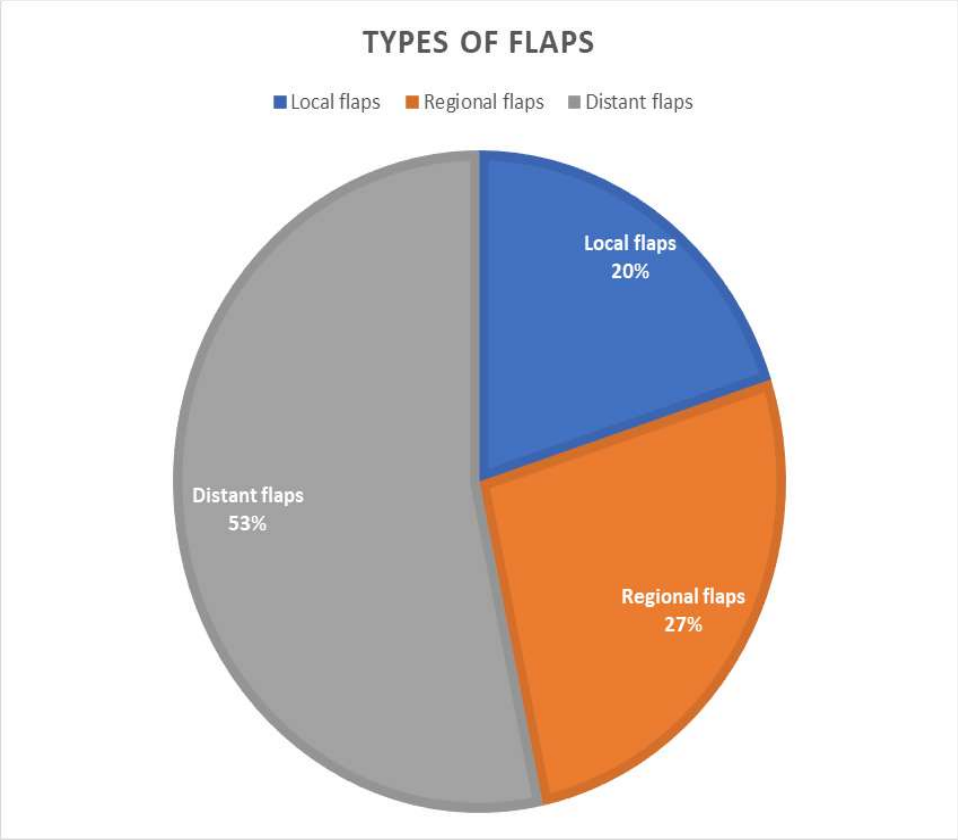

Figure 9: Distribution of flaps

8. Planned flap area

In this study, a total of 30 flaps were part of the study. These amounted to 3305 cm^2 in total with an average flap area of 110.12 cm^2.

The largest planned flap was a pedicled latissimus dorsi flap (Case No 22) measuring 400 cm^2. The smallest planned flap area was 24 cm^2 for a split abdominal flap (Case No. 6)

Table 8: Total planned flap area

Type of flap	Total flap area (cm^2)
Right groin flap	80
Left groin flap	210
Right PMMC flap	60
Left PMMC flap	50
Right medial gastrocnemius flap	225
Split abdominal flap	24
Left PMMC flap	60
Left radial artery free flap	119
LD free flap	252
ALT fasciocutaneous free flap	180
Reverse sural artery flap	40
Fasciocutaneous rotational advancement flap	60
Right groin flap	160
Cross leg flap	60
Right medial gastrocnemius	126
Leg transposition flap	136
Mustarde advancement flap	36
Leg Transposition flap	105
Left radial artery free flap	54
Right radial artery free flap	49
Cross leg flap	170
Pedicled LD flap	400
Cross leg flap	60
Right groin flap	60
ALT fasciocutaneous flap	120
Leg transposition flap	40
Right groin flap	160
Cross leg flap	50
Reverse sural artery flap	34
Leg transposition flap	125

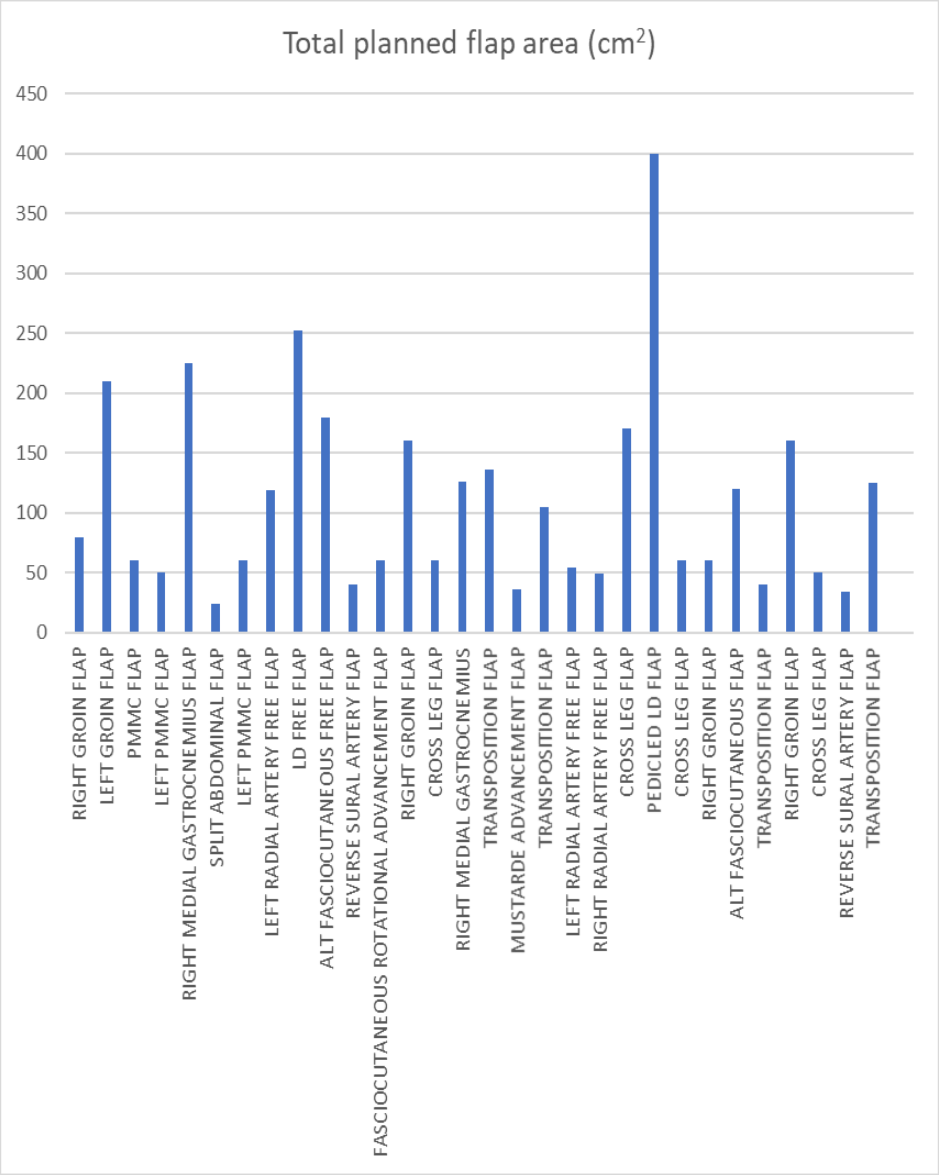

Figure 10: Total planned flap area

9. **Intraoperative clinical flap asessment vs. Intraoperative fluorescein dye flap assessment.**

In 7 cases there was a difference in flap viability when intraoperative clinical assessment was compared with fluorescein dye assessment. This difference was as high as 60 cm^2 to as low as 4 cm^2 across different flaps.

The average flap area deemed to be viable after intraoperative clinical examination was 107.83 cm^2 compared to intraoperative fluorescein dye assessment of 103.37 cm^2 in our study.

In no case was the area of flap deemed viable via fluorescein greater than the area deemed viable by intraoperative clinical assessment.

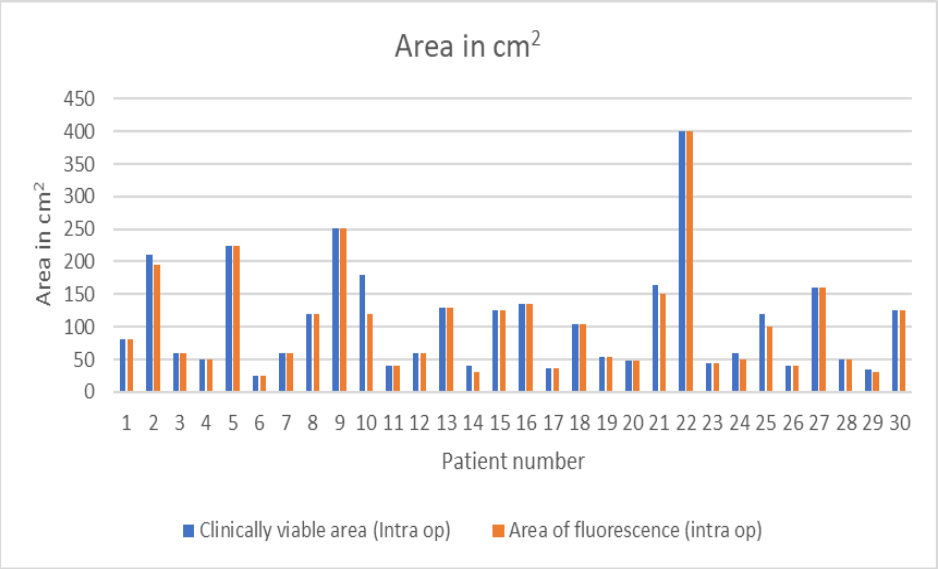

Figure 11: Intraoperative clinical flap assessment versus Fluorescein dye flap assessment

On statistical analysis, p - value was less than 0.05, which *was statistically significant.*

Table 9: Intraoperative clinical flap assessment versus Fluorescein dye flap assessment

	Mean	Std. Deviation	Std. Error Mean	Z	p-value	Difference		95% Confidence Interval of the Difference	
						Mean	Std. Deviation	Lower	Upper
Clinically viable area (Intraop)	107.83	83.47	15.24	-3.066	0.002	4.47	11.84	0.04	8.89
Area of fluorescence	103.37	82.02	14.98						

10. Intraoperative fluorescein dye flap assessment vs clinical flap assessment on post-operative day 5

The average flap area deemed to be viable when assessed with fluorescein dye was 103.37 cm^2 as compared to clinical flap assessment on day 5 which was 103.20 cm^2 in our study. In this study, there were only 3 cases where there was a difference between intraoperative fluorescein dye assessment and post-operative assessment done at day 5 (Case no. 2, case no. 14 and case no. 21)

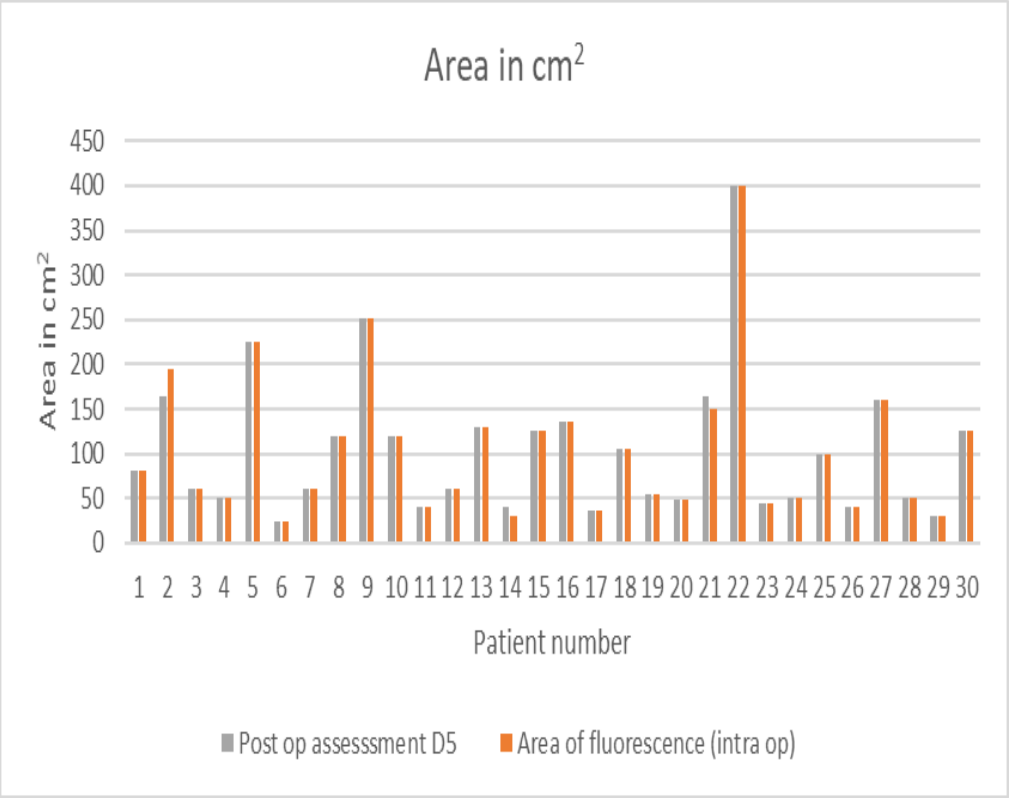

Figure 12: Intraoperative fluorescein dye flap assessment vs clinical flap assessment on Post-operative day 5

On statistical analysis, the p -value was greater than 0.05, which was not *statistically significant*.

Table 10: Intraoperative fluorescein dye flap assessment vs clinical flap assessment on Post-operative day 5

	Mean	Std. Deviation	Std. Error Mean	Z	p-value	Difference		95% Confidence Interval of the Difference	
						Mean	Std. Deviation	Lower	Upper
Area of fluorescence	103.37	82.02	14.98	-1.342	0.180	0.17	6.50	-2.26	2.59
Post op assessment D5	103.20	81.11	14.81						

11. **Intraoperative clinical flap assessment vs clinical assessment performed on day 5**

The average flap area deemed to be viable when assessed with intra operative clinical assessment was 107.83 cm^2 as compared to clinical flap assessment on day 5 which was 103.20 cm^2 in our study. In this study, there were 5 cases where there was a difference between intraoperative clinical assessment and post-operative assessment done at day 5 (Case no. 2, case no. 10, case no 24, case no. 25 and case no. 29)

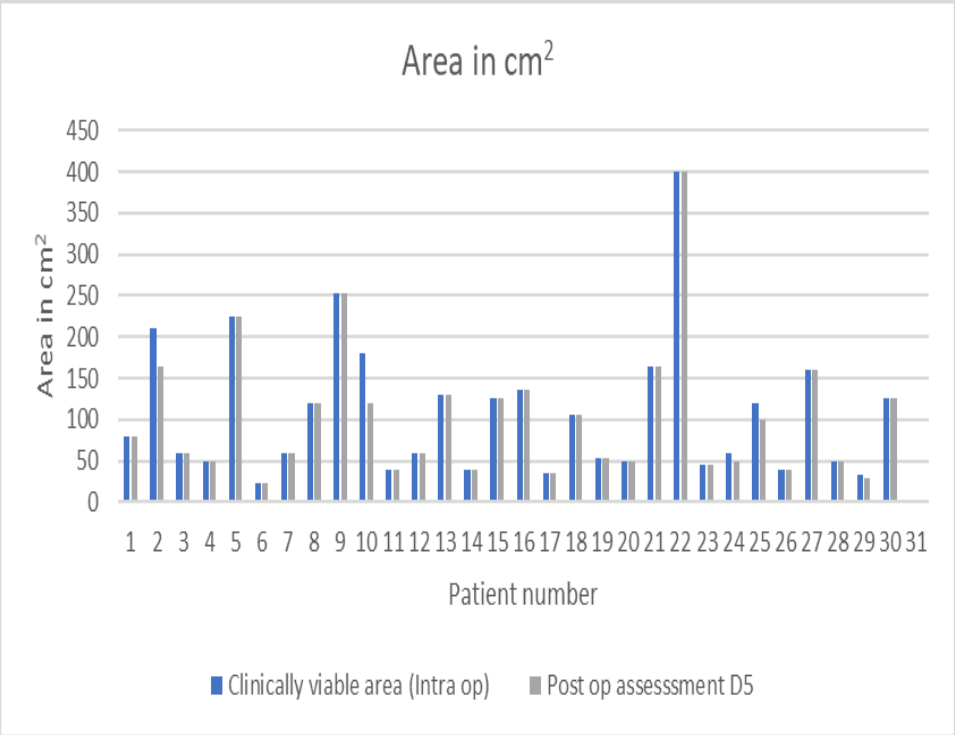

Figure 13: Intraoperative clinical flap assessment vs clinical assessment performed on day 5

On statistical analysis, the p -value was less than 0.05, which was *statistically significant*.

Table 11: Intraoperative clinical flap assessment vs clinical assessment performed on day 5

	Mean	Std. Deviation	Std. Error Mean	Z	p-value	Difference		95% Confidence Interval of the Difference	
						Mean	Std. Deviation	Lower	Upper
Clinically viable area (Intraop)	107.83	83.47	15.24	-3.193	0.001	4.63	13.77	-0.51	9.77
Post op assesssment D5	103.20	81.11	14.81						

12. Individual case analysis

In this study there were 7 cases where there were differences between intraoperative assessment (fluorescein & clinical assessment) and post-operative clinical assessment. On further analysis the following was observed.

In 4 out of the 7 cases (case no. 10, 24, 25 and 29), there was no difference between intraoperative fluorescein assessment and post-operative clinical assessment on day 5.

In 2 of the other 3 cases (case no 14 and 21), there was no difference between clinical assessment of flap done intraoperatively and at post-operative day 5.

In only one case (case no. 2) was there a significant difference across all three parameters.

Table 12: Individual case analysis

Case No.	Clinically viable area (Intra op)	Area of fluorescence (intra op)	Post op assessment D5
2	210	195	165
10	180	120	120
14	40	30	40
21	165	150	165
24	60	50	50
25	120	100	100
29	34	30	30

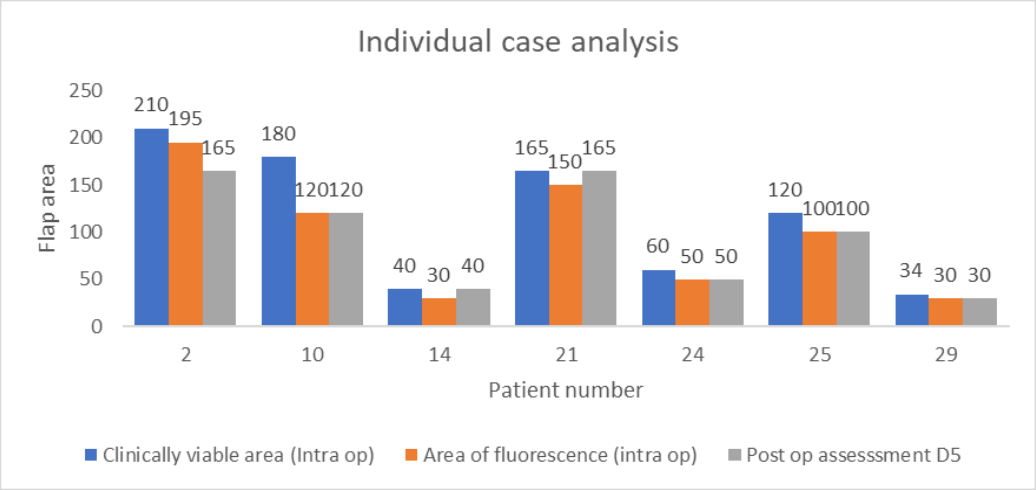

Figure 14: Individual case analysis

13. Complications

In this study, complications were seen in 8 cases. The most common complication seen was marginal necrosis seen in 5 cases, followed by SSI seen in 2 cases.

Table 13: Complications

Complications	No. of cases
SSI	2
Haematoma	1
Marginal necrosis	5

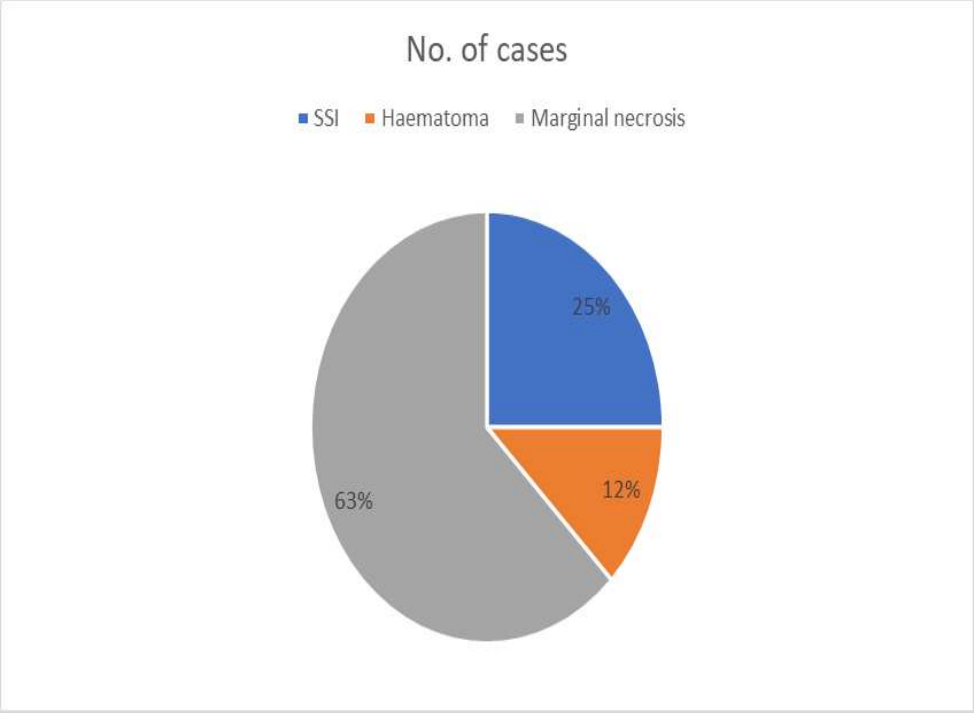

Figure 15: Complications

DISCUSSION

Monitoring of flaps has been a difficult undertaking. Over the years numerous methods have been published in search of the ideal technique. Surgeons rely on clinical examination to assess the viability of flaps by measuring temperature, colour, pin prick, capillary refill & skin turgor. On table assessment by clinical methods can often be misleading due to the presence of blood in the operative field and heat generated by lights and body warmers. Under these circumstances objective measures of flap assessment are necessary.

In this prospective study, we used Fluorescein dye as an adjunct to assess its use for perioperative flap viability. We evaluated 30 patients operated for various defects requiring flap coverage. The effects of the dye were assessed intraoperatively and areas of fluorescence and non-fluorescence were marked onto a graph paper. A similar assessment was done using clinical examination intraoperatively and on post-operative day 5. These assessments were marked onto a graph paper as well.

It has been previously suggested that use of Fluorescein dye is an accurate method for assessment of flap viability.[11,13,18,19,43,60, 75,76,79,81,82]

Age

The mean age of our study group is 39.43 years. A similar study by Losken et al, had an average age of 50 years.[11]

Sex

In our study, there was a predominance of male patients who comprised 73.3% of all cases and 26.7% cases were females. In the study by Bhattacharya et al., males comprised of 83.3 % of their study group.[18] Losken et al. had a study group comprising exclusively of female patients.[11]

Etiology

In this study, trauma was the most common etiology of the defect accounting for 19 cases (63.33%), followed by cancer accounting for 8 cases (26.6 %). In the study by Losken et al. cancer of the breast

constituted 100% of their study group.[11] The study by Bhattacharya et al. had defects over ankle, heel and unstable scar of the foot as the most common etiology, accounting for 33.3% of their study group.[18]

Anatomical site of defect

In our study, the lower limb was the most common site of the defect (46.4%), followed by the head and neck region (30%) and the upper limb (20%). In the study by Bhattacharya et al., the most common site of defect was the lower limb in 90 % of cases[18]. In the study by Losken, the breast was the only site of the defect.[11]

Type of flap

The groin flap was the most common flap performed accounting for 16.7% of all cases, followed by the cross leg and transposition flap (13.3% each). In the study by Bhattacharya et al., Fasciocutaneous flaps constituted 53.3% of all flaps followed by adipofascial flaps which constituted 20% of all flaps.[18] In the study by McCraw et al., the most common flap was the facial axial cutaneous flap (20%), followed by the Gracilis myocutaneous flap (16.84%).[19]

Assessment of flap viability

In 23 out of 30 cases, the area of clinically assessed viable flap was equal to viable flap area as determined by fluorescein dye. We observed that there was a difference in flap viability when intraoperative clinical assessment was compared with intra operative fluorescein dye assessment in 7 cases. The difference was as high as 60 cm^2 to as low as 4 cm^2. The average flap area deemed to be viable after intraoperative clinical examination was 107.83 cm^2 compared to intraoperative fluorescein dye assessment of 103.37 cm^2 in our study. On average we found that the area deemed to be viable was 4.47 cm^2 lower when assessed with the Fluorescein dye. The difference was statistically significant (p value = 0.002). We observed that in 27 out of 30 cases the viable flap area on post-operative day 5 was equal to the staining area as predicted by fluorescein dye given intraoperatively. Thus, having a giving positive predictive value of 90% (n=27/30). The average flap area which was deemed viable after clinical assessment on day five was 103.20 cm^2. The area deemed viable after

intraoperative fluorescein dye was 103.37 cm^2. The difference was not statistically significant (p-value = 0.180), implying that the areas showing fluorescence intraoperatively were similar to the flap area assessed on post-operative day 5.

In our study, the average flap area deemed to be viable when assessed with intra operative clinical assessment was 107.83 cm^2 as compared to clinical flap assessment on day 5 which was 103.20 cm^2. In the five cases where there was a difference between the two assessments, the area deemed viable on intraoperative assessment was always higher. The difference was statistically significant (p-value= 0.01).

On individual case analysis we discovered that when the three values of viable tissue area were compared head-to-head, in 23 cases, the value of all three variables was equal and there were differences in 7 cases (cases 2, 10, 14, 21, 24, 25 and 29). Of these, in 4 out of the 7 cases fluorescein dye accurately predicted the fate of the flap as judged clinically on post-operative day 5. In 2 cases (case no 14 and 21), fluorescein seemed to underestimate, the eventual area of viable tissue. There was only one case, where the value across all three measurements was different – case no. 2. However, even in that case, the eventual area of viable tissue (165 cm^2) was closer to the estimate provided by intraoperative fluorescein (195 cm^2) than intraoperative clinical examination.

This implies that if we were to excise all tissue that did not show fluorescence intraoperatively, we would have 99.03 % flap area survival on post-operative day 5, as opposed to a 95.70 % flap area survival based on the intraoperative clinical assessment.

In our study, we thus conclude that fluorescein dye remains a valuable tool to augment clinical observation at the time of flap raising but underestimates flap viability in a few cases.

Losken et al. had similar results and suggested that fluorescein is a sensitive test for determining native skin flap viability, but underpredicts flap viability.[11] In two of our cases, fluorescein dye underreported the amount of eventual flap survival. The percentage of flap area

underreported by the fluorescein dye was, 25% and 9.09% for case no. 14 and 21 respectively.

Our results are similar to those of Myers et al. who concluded that the test is a reliable test of vascularity. In their study, the mean difference of flap survival and fluorescein dye estimate was 0.02 cm, compared to mean difference of survival of 0.017 cm in our study. In their study they also reported that the dye was occasionally under reading by as much as 3.5 cm. These changes were attributable by the authors to vasospasm, intravascular clotting or alterations in the distribution of the microcirculation.[20]

Myers et al. performed another experiment where they discovered that fluorescein given intraoperatively was an accurate predictor of flap survival when measured on post-operative day 7. The mean difference between intra operative dye assessment and post-operative assessment in their study was 0.1 cm.[83]

Bardach and Panje explored the difference between staining and surviving flap area after the instillation of Fluorescein dye. In their experimental study on rats, they discovered that there was a significant difference between the staining and surviving areas of unipedicled flaps with the surviving area being as great as 31% more than the staining area. Their study suggested that the fluorescein dye is not an accurate predictor of flap survival, with surviving area being greater than dye staining area.[76] This is in contrast to our study as the dye was accurate at predicting viable flap area at post-operative day 5 with an accuracy of 99.03 %. Dibbell et al. had similar results from their experiment, with the fluorescein dye having predicted flap viability of 99.33 %.[13] Bhattacharya et al. reported a 100% rate of accuracy, with the use of the fluorescein dye. However, in contrast to our study, they injected fluorescein dye 20 minutes after the flap was raised, and waited a further 20 minutes to observe the dye under UV light. The authors suggested that this time period was appropriate to overcome the initial vasospasm caused by dissection of the flaps.[18]

McCraw et al. in their study of axial and myocutaneous flaps concluded that the test predicts flap viability when properly interpreted with an

error of ~ 3 to 5 mm per flap. The authors also observed that capillary refill and flap colour were questionable measures of flap viability with marked variations in various flaps that were raised in their study. Based on their results, the authors concluded that fluorescein deposition was an apt test for assessing functional microcirculation.[19]

Reinisch's experiments with fluorescein and radioactive microspheres discovered that there was an abrupt interface between fluorescein staining and non-staining areas. He suggested that circulation within a flap terminates at a point. The simplest explanation for this could come from the pharmacokinetics of Fluorescein dye. To be visualized, the dye must diffuse into the interstitial fluid. It is conceivable that in non-staining areas which survive, circulation is present without capillary flow. This can be accounted for by opening up of arteriovenous shunts. Like capillaries, these structures connect the arterial and venous circulations. However, owing to their thick walls, the do not allow metabolic exchange. Thus, circulation could exist via these low resistance arteriovenous channels without causing tissue staining.[84] Newer dyes like Indocyanine Green are bereft of these issues, owing to their larger size and predominant intravascular distribution, but their widespread use is limited by the need for special equipment and high cost of use.[74] .Dibbell et al. also suggested that the distal section of flaps receive some nutrition from the wound bed where they are placed, thus acting like a graft. In this scenario, the distant non fluorescent areas which would be destined for necrosis, would still survive.[13] This would explain why in two of our flaps, the dye under reported the amount of final surviving tissue.

Complications.

Thorvaldsson and Grabb, in their study on 19 patients observed that in 5 flaps, there were non fluorescing areas at the distal end leading to eventual necrosis. In their study, they had distal necrosis in 26.16 % of their study sample, compared to 16.6% in our study. The authors concluded that fluorescein dye accurately predicted eventual flap necrosis in 100% of these cases.[75] Myers et al. reported that in one of their studies, fluorescein dye predicted flap necrosis with an accuracy

of 85.71%.[85] In their study, Losken et al., had skin necrosis in 5 out of 21 non fluorescing flaps. Their study had a negative predictive value of 25%.[11] Thorvaldsson and Grabb, in their study had a negative predictive value of 100%.[75] In our study, marginal necrosis was seen in 5 patients. Fluorescein dye accurately predicted marginal necrosis with negative prediction rate of 80% of the time (n = 4). No anaphylactoid reactions were observed in our study.

Despite its limitations, we conclude that the Fluorescein dye has a role to play in the assessment of flap surgery, when used immediately after raising a flap. If the raised flap is non fluorescent, the operating surgeon can either resect the non-viable part of the flap, or use pharmacological measures to salvage the predicted area of necrosis. These measures may include agents like Dextran, Deferoxamine, Phenoxybenzamine, Phentolamine, Enalapril, Statins and Sildenafil amongst other pharmacological measures designed to increase flap viability.[86-91] In certain flaps, the surgeon may choose to perform a delay, in order to ensure robustness of the vascularity of the flap and confirm vascularity by reperforming the Fluorescein test at the time of the final surgery. Since the dye spreads to the flap in its entirety, the whole flap can be visualized and is thus not subject to sampling error.

Although, the Fluorescein dye test is a reliable, rapid, cost effective and objective test to assess for flap viability, its use for flap monitoring should be as an adjunct to clinical examination which remains the gold standard for flap monitoring.

SUMMARY AND CONCLUSION

Accurate assessment of flap viability has been an area of constant research. One of the methods described has been the use of various dyes given at the time of flap raising. Fluorescein has been used in numerous animal and human studies to elucidate tissue vascularity.

Use of intraoperative fluorescein dye for prediction of flap viability has an accuracy of 99.03% compared to intraoperative clinical flap assessment accuracy of 95.70%. From this study, we conclude that using the Fluorescein dye as a predictor of flap viability is accurate when used immediately after raising the flap, but underestimated flap viability in a few cases. When compared to clinical examination, fluorescein dye given intraoperatively accurately predicted flap viability.

Although the results of our study are in favour of using Fluorescein dye at the time of raising the flap, our study was limited by a small sample size, varied distribution of diagnosis and lack of comparison to other known objective methods. We opine that the Fluorescein dye may corroborate the clinical assessment of viable flap tissue as assessed by a surgeon intraoperatively. More powerful randomized clinical trials and meta-analysis are needed to accept or deny the role of Fluorescein dye to predict flap viability.

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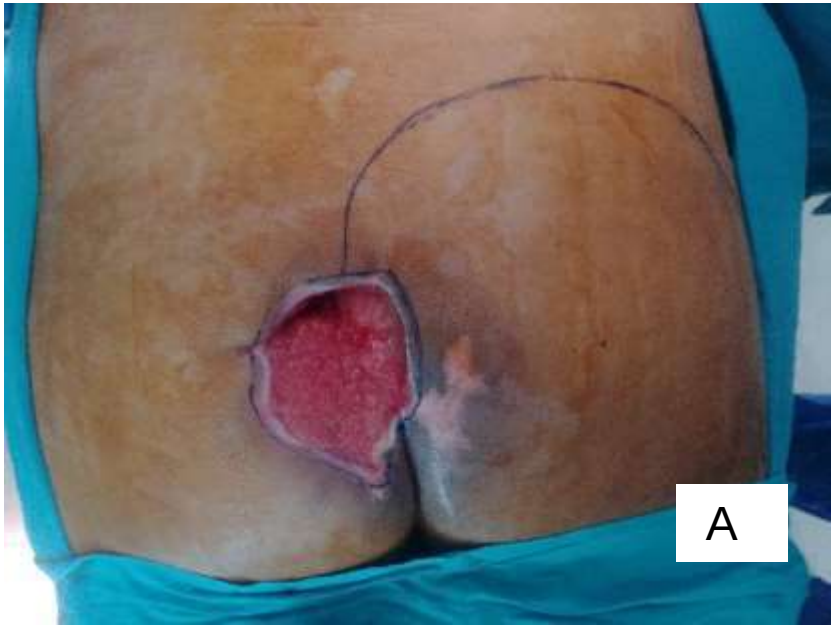
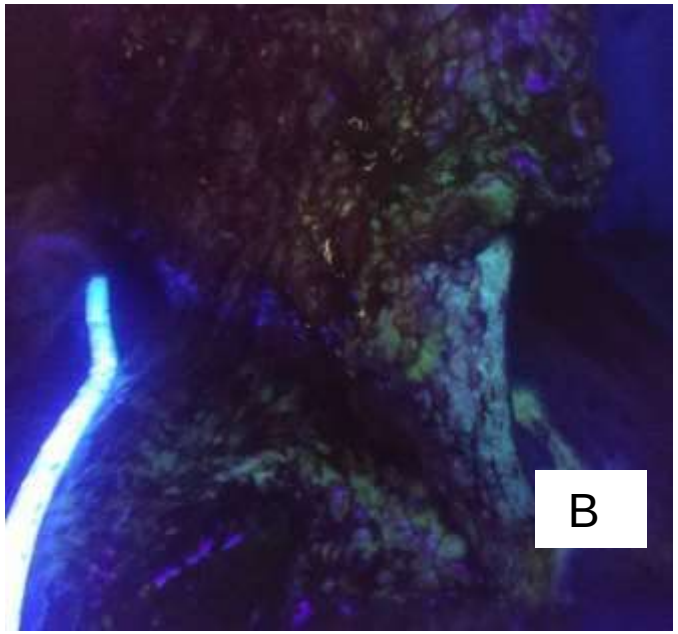
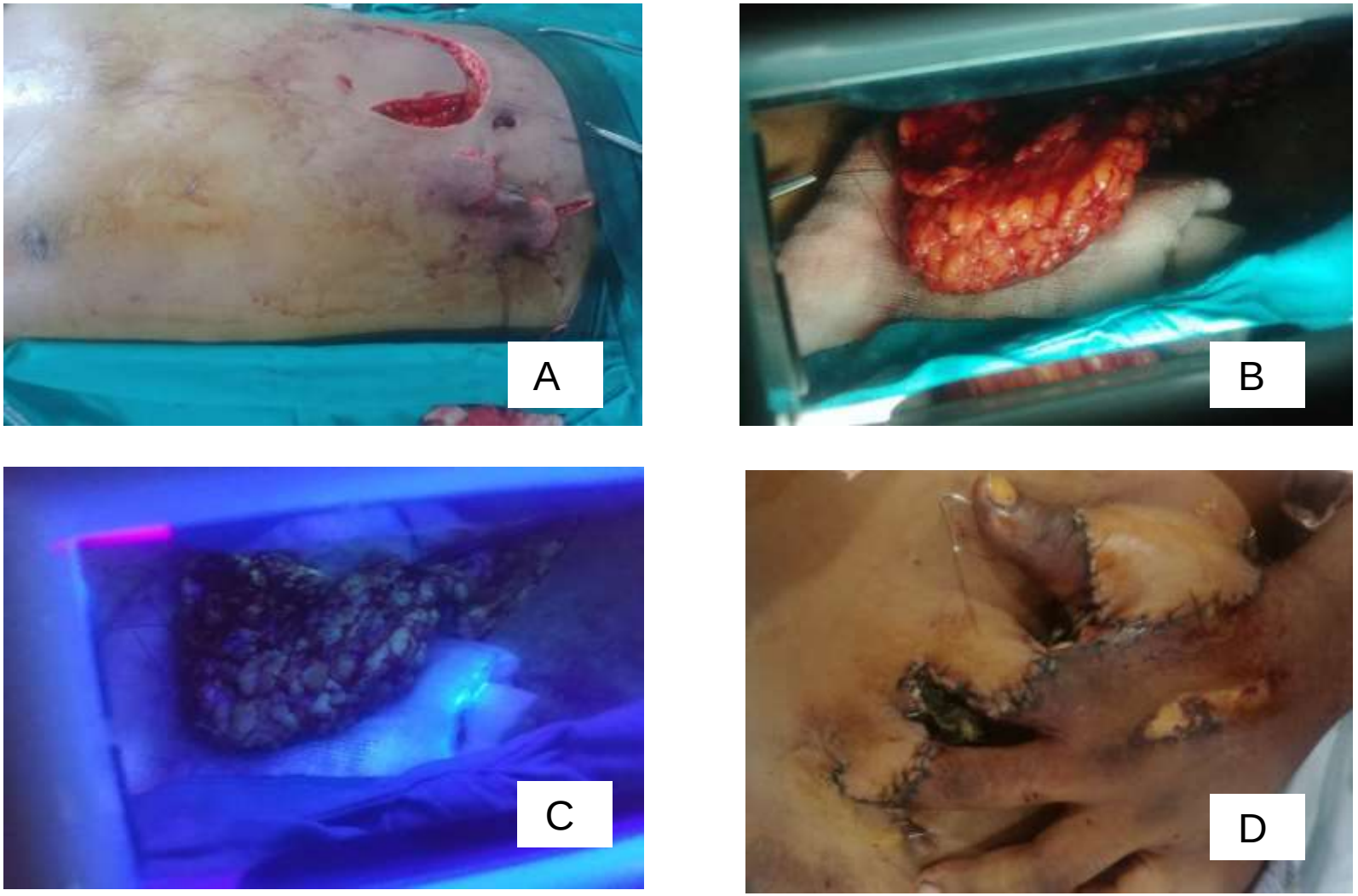
Picture 1. Injection Fluorescein

Picture 2. Wood's Lamp

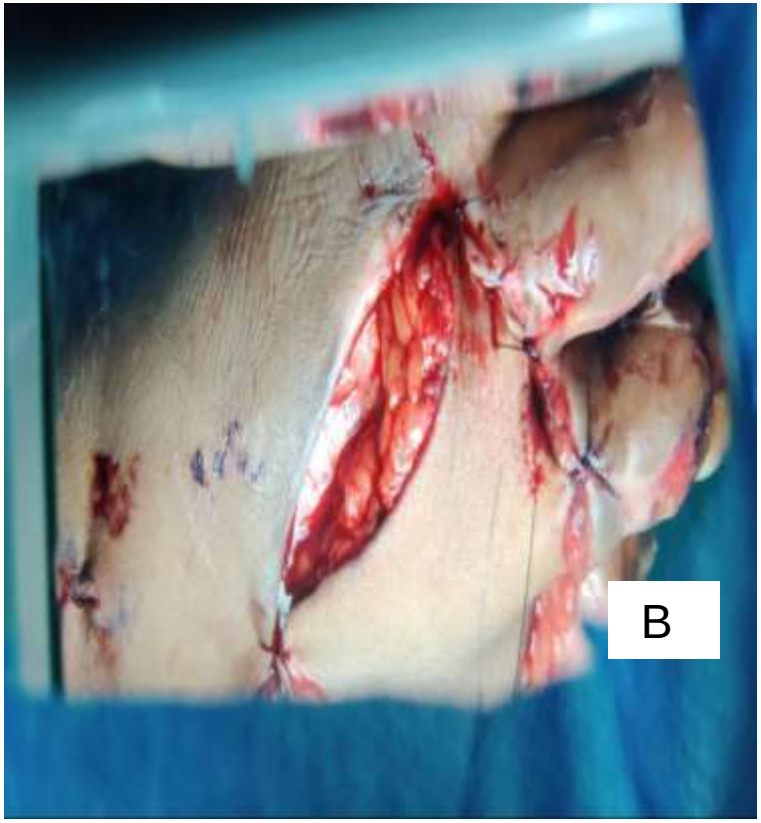




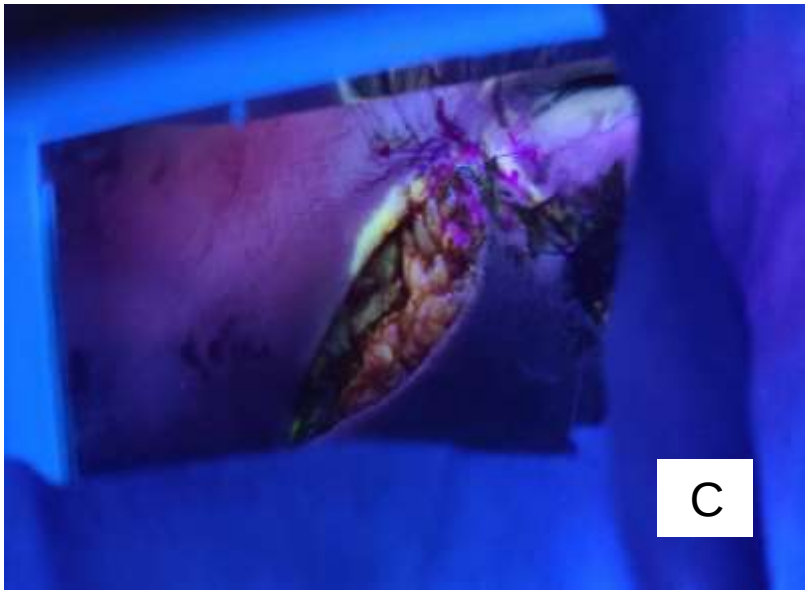
Picture 3. (A) Sacral bed sore defect with marking of rotation advancement Flap (B) Intra operative Fluorescein dye visualized under UV light at distal flap margin (C) Flap after inset.



Picture 4. (A) Raised abdominal flap (B) Flap visualized in normal light intra operatively (C) Fluorescein dye visualized under UV light in flap intra operatively (D) Flap after inset



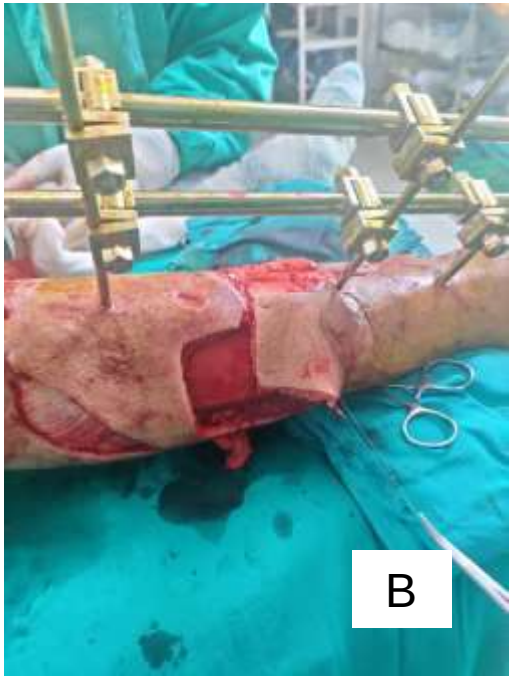
Picture 5a. (A) Right forefoot defect (B) Flap visualized in normal light



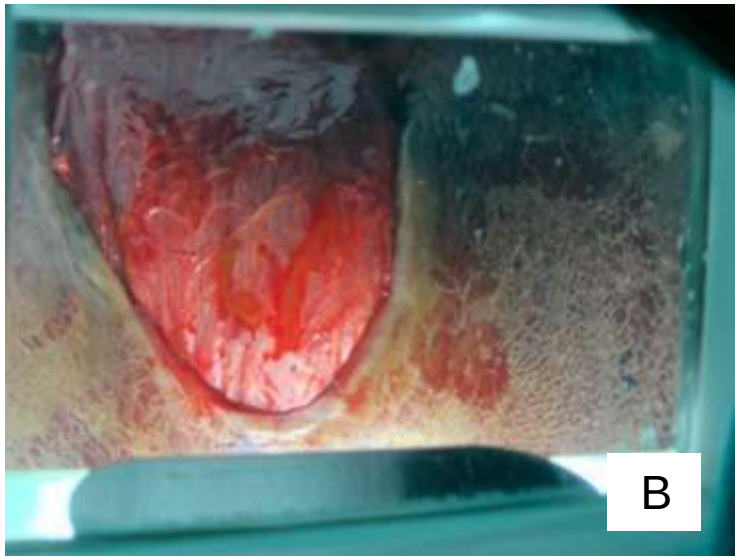
Picture 5b (C) Fluorescein dye visualized under UV light intra operatively (D) Partially necrosed flap seen at post operative day 5

Picture 6. (A) Defect left hand (B) Raised left groin flap (C) Flap after inset

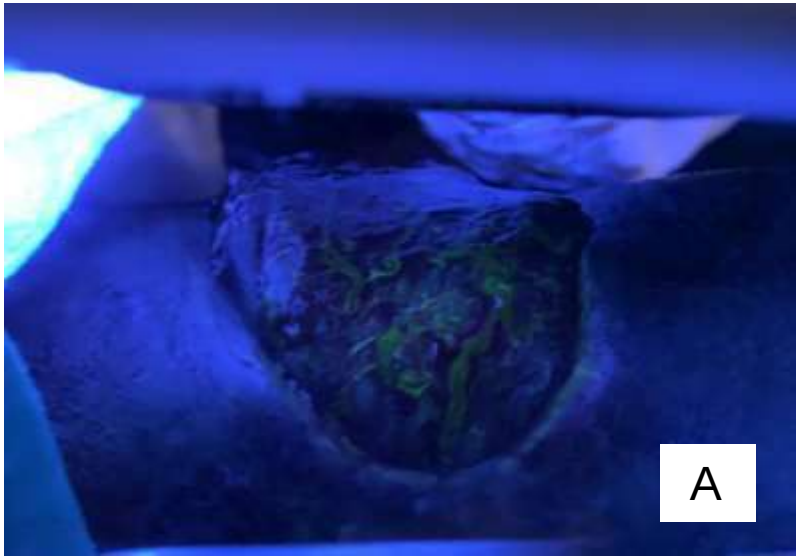
Picture 7. (A) Defect Right leg (B) Transposition Flap after inset



Picture 8. (A) Defect Right leg (B) Flap raised (C) Transposition Flap after inset



Picture 9a. (A) Defect Right leg (B) Flap seen under normal light intra operatively.



Picture 9b (C) Fluorescein dye visualized under UV light intra operatively (D) Medial Gastrocnemius flap after inset

Picture 10a. (A) Defect left buccal mucosa (B) Flap seen under normal light intra operatively.

Picture 10b. (C) Fluorescein dye visualized under UV light intra operatively
(D) Pectoralis Major flap after inset

S No	Name	Age	Sex	Body Weight	Diagnosis	Type of flap
1	AK	30	F	45	RIGHT DORSUM HAND DEFECT	RIGHT GROIN FLAP
2	NS	33	F	52	POST BURN CONTRACTURE LEFT UPPER LIMB	LEFT GROIN FLAP
3	RS	42	M	54	RIGHT CA BUCCAL MUCOSA	PMMC FLAP
4	HM	64	F	32	RECURRENT CA UPPER ALVEOLUS LEFT SIDE	LEFT PMMC FLAP
5	MS	23	M	56	RIGHT LEG UPPER 1/3RD DEFECT	RIGHT MEDIAL GASTROCNEMIUS FLAP
6	BS	21	M	60	POST BURN RAW AREA LEFT HAND	SPLIT ABDOMINAL FLAP
7	AP	62	M	70	LEFT CA BUCCAL MUCOSA	LEFT PMMC FLAP
8	NK	57	M	60	LEFT CA BUCCAL MUCOSA	LEFT RADIAL ARTERY FREE FLAP
9	V	27	M	65	LEFT ANTEROLATERAL THIGH DEFECT	LD FREE FLAP
10	P	25	M	52	POST ELECTRIC BURN RIGHT FOREFOOT DEFECT	ALT FASCIOCUTANEOUS FREE FLAP
11	NS	40	M	56	LEFT HEEL DEFECT	REVERSE SURAL ARTERY FLAP
12	KS	70	M	90	STAGE 4 SACRAL PRESSURE SORE	FASCIOCUTANEOUS ROTATIONAL ADVANCEMENT FLAP
13	AK	38	M	64	RIGHT HAND DEGLOVING INJURY	RIGHT GROIN FLAP
14	MK	20	M	45	RIGHT DORSUM FOOT DEFECT	CROSS LEG FLAP
15	S	30	M	54	RIGHT UPPER 1/3RD LEG DEFECT	RIGHT MEDIAL GASTROCNEMIUS
16	BK	54	F	50	POST RSA RIGHT LEG INFECTED WOUND	TRANSPOSITION FLAP
17	CK	71	F	65	BCC LEFT CHEEK	MUSTARDE ADVANCEMENT FLAP
18	RS	20	M	46	SOFT TISSUE DEFECT LEFT MASTOID AND LEFT LEG	TRANSPOSITION FLAP
19	MS	34	M	53	CA LOWER LIP	LEFT RADIAL ARTERY FREE FLAP
20	GS	24	M	45	LEFT CA BUCCAL MUCOSA	RIGHT RADIAL ARTERY FREE FLAP
21	SK	56	M	60	RIGHT MIDDLE 1/3RD LEG DEFECT	CROSS LEG FLAP
22	ON	65	M	65	CA RIGHT BUCCAL MUCOSA AMD CHEEK	PEDICLED LD FLAP
23	AK	25	F	50	LEFT LOWER 1/3RD LEG DEFECT	CROSS LEG FLAP
24	BS	36	M	54	RIGHT VOLAR FOREARM DEFECT	RIGHT GROIN FLAP
25	DP	35	M	50	POST ELECTRIC BURN SCALP DEFECT	ALT FASCIOCUTANEOUS FLAP
26	AT	26	F	46	LOWER 1/3RD LEG DEFECT	TRANSPOSITION FLAP
27	GS	33	M	72	RIGHT HAND DEGLOVING INJURY	RIGHT GROIN FLAP
28	MR	58	M	67	RIGHT MIDDLE 1/3RD LEG DEFECT	CROSS LEG FLAP
29	AG	42	F	57	RIGHT HEEL DEFECT	REVERSE SURAL ARTERY FLAP
30	S	22	M	58	LEFT LEG LOWER 1/3RD DEFECT	TRANSPOSITION FLAP

S No	Name	Age	Anatomical location	Total flap area cm^2	Clinically viable area (Intra op) cm^2	Area of fluorescence (intra op) cm^2	Post op assessment D5 cm^2	Comparison with Fluorescein cm^2	Complications
1	AK	30	RIGHT GROIN TO RIGHT DORSUM HAND DEFECT	80	80	80	80	0	NO
2	NS	33	LEFT GROIN TO LEFT DORSUM LEFT HAND	210	210	195	165	15	MARGINAL NECROSIS
3	RS	42	RIGHT BUCCAL MUCOSA AND CHEEK	60	60	60	60	0	SSI
4	HM	64	ORAL CAVITY AND BUCCAL MUCOSA	50	50	50	50	0	NO
5	MS	23	RIGHT UPPER 1/3RD LEG	225	225	225	225	0	NO
6	BS	21	ABDOMEN	24	24	24	24	0	NO
7	AP	62	LEFT BUCCAL MUCOSA AND NECK	60	60	60	60	0	NO
8	NK	57	LEFT BUCCAL MUCOSA AND CHEEK	119	119	119	119	0	NO
9	V	27	LEFT ANTEROLATERAL THIGH	252	252	252	252	0	NO
10	P	25	RIGHT FOREFOOT	180	180	120	120	60	MARGINAL NECROSIS
11	NS	40	LEFT HEEL	40	40	40	40	0	NO
12	KS	70	SACRAL SORE	60	60	60	60	0	NO
13	AK	38	RIGHT GROIN TO PALMER SURFACE OF HAND	160	130	130	130	0	NO
14	MK	20	LEFT LEG TO RIGHT FOOT	60	40	30	40	10	NO
15	S	30	RIGHT LEG	126	126	126	126	0	NO
16	BK	54	RIGHT LEG	136	136	136	136	0	NO
17	CK	71	LEFT CHEEK	36	36	36	36	0	NO
18	RS	20	LEFT LEG	105	105	105	105	0	NO
19	MS	34	LEFT FOERARM OF LOWER LIP	54	54	54	54	0	NO
20	GS	24	LEFT BUCCAL CAVITY	49	49	49	49	0	NO
21	SK	56	LEFT TO RIGHT LEG	170	165	150	165	15	HEMATOMA
22	ON	65	RIGHT BUCCAL MUCOSA AND CHEEK	400	400	400	400	0	NO
23	AK	25	RIGHT TO LEFT LEG	60	45	45	45	0	NO
24	BS	36	RIGHT GROIN TO RIGHT FOREARM	60	60	50	50	10	MARGINAL NECROSIS
25	DP	35	LEFT THIGH TO SCALP	120	120	100	100	20	MARGINAL NECROSIS
26	AT	26	RIGHT LEG	40	40	40	40	0	NONE
27	GS	33	RIGHT GROIN TO RIGHT HAND	160	160	160	160	0	SSI
28	MR	58	LEFT TO RIGHT LEG	50	50	50	50	0	NONE
29	AG	42	RIGHT HEEL	34	34	30	30	4	MARGINAL NECROSIS
30	S	22	LEFT LEG	125	125	125	125	0	NONE

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No. BFUHS/2K19p-TH/ ۱۰۲۳۵

Date ۱۳/۶/۱۹

The Principal,
DMC, Ludhiana.

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 29.05.2019 has approved the plan of thesis of **Dr. Tushar Patial**, a student of your college for **MCh Plastic & Reconstructive Surgery** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis

Use of fluorescein dye for perioperative flap viability

Supervisor
Dr. Rajinder K. Mittal
Prof.,
Dept. of Plastic Surgery,
DMCH, Ludhiana.

Co-Supervisor
Dr. Sanjeev K. Uppal
Prof. & Head,
Dept. of Plastic Surgery,
DMCH, Ludhiana.

The student has to submit the four copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

S P Singh

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

USE OF FLUORESCEIN DYE FOR PERIOPERATIVE FLAP VIABILITY

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF
MCh

in

Plastic and Reconstructive surgery

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

APRIL, 2019

DR TUSHAR PATIAL

DEPARTMENT OF PLASTIC AND RECONSTRUCTIVE SURGERY
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	Use of Fluorescein dye for perioperative flap viability
For the degree of	MCh
Name of the candidate	Dr Tushar Patial
Supervisor	Dr Rajinder K Mittal, Professor, Department of Plastic Surgery, DMCH, Ludhiana
Co-supervisor	Dr Sanjeev K Uppal, Professor and Head, Department of Plastic Surgery, DMCH, Ludhiana
Institution	Dayanand Medical College & Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: A successful flap is dependent on its vascularity. During dissection, flap viability may become compromised due to inadequate perfusion from the vascular pedicle(s) on which the flap is based. Various methods have been proposed to determine vascularity of a flap both, intraoperatively and postoperatively. The ideal method must be simple, accurate, reproducible and safe.

Aim of Study: This study deals with the use of fluorescein dye to evaluate the vascularity of various flaps.

Materials & Methods: The prospective study will be carried out on 30 patients undergoing flap coverage for various defects. Intravenous fluorescein shall be injected into the patients and its course through the flap will be monitored under ultraviolet light. Areas of reduced perfusion shall be marked and assessed periodically.

Clinical Significance: The usage of fluorescein dye may provide an easy method to determine flap viability during surgery. If successful, it may be found to be a safe and reliable method to determine adequacy of perfusion during flap surgery.

Keywords: Fluorescent Dyes, Surgical Flaps, Intraoperative Care, Ultraviolet Rays.

(Signature)	(Signature)	(Signature)	(Signature)
Candidate	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"USE OF FLUORESCEIN DYE FOR PERIOPERATIVE FLAP VIABILITY"** exist in the Department of Plastic Surgery, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate himself under our supervision and guidance.

Supervisor

Dr Rajinder K Mittal
Professor
Department of Plastic Surgery
DMCH, Ludhiana

Co-Supervisors

Dr. Sanjeev K Uppal
Professor & Head
Department of Plastic Surgery
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Head of the Department

Dr. Sanjeev K Uppal
Professor & Head
Department of Plastic Surgery
DMCH, Ludhiana

Place : Ludhiana

Date : 18/4/19

APPROVAL PROFORMA
BY
RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr Tushar Patial
Department	Plastic and Reconstructive Surgery
Topic of Thesis	Use of Fluorescein dye for perioperative flap viability
Likely date of appearing in PG examination	May 2021
Date of enrollment	26th August, 2018
Name of Head of Department	Dr. Sanjeev K Uppal
1) Supervisor **2) Co-Supervisor(s)**	Dr. Rajinder K Mittal Dr. Sanjeev K Uppal
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
1. HOD Pharmacology	1. HOD Physiology
2. HOD Surgery	2. HOD Pathology
3. HOD Pathology	3. HOD Pharmacology
4. HOD Medicine	4. HOD SPM
5. Dean Academics	5. HOD Medicine
6. Vice Principal	6. HOD Surgery
	7. HOD Obst. & Gynae
	8. Legal Expert
	9. Secretary, IEC
	10. Dean Academics
	11. Vice Principal
	12. Principal

Approved: Yes / No **Approved: Yes / No**

Chairperson
Research Committee

Chairperson
Ethics Committee

Chairperson
Research Committee

Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	Dr Tushar Patial, Rameshwaram, Nav Bahar, Near Motoworld, Navbahar, Shimla, Himachal Pradesh, 171002
2	NAME OF THE INSTITUTE	Dayanand medical college and hospital, Ludhiana
3	COURSE OF STUDY AND SUBJECT	MCh, Plastic and Reconstructive surgery
4	DATE OF ADMISSION OF COURSE	26 August, 2018
5	TITLE OF THE TOPIC	Use of Fluorescein dye for perioperative flap viability

6 BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Flap reconstruction is used for coverage of defects throughout the various parts of the body. However, despite best efforts, flap necrosis remains a dreaded complication of the surgery. Identification of adequate vascularity is paramount to prevent this complication. When a suspicion of flap compromise is suspected, immediate interventions can help salvage the flap and/or revise the surgical plan to undertake some other form of reconstruction. Various methods of measuring flap viability have been proposed to recognize flap compromise as soon as possible.

Surgeons rely on physical examination to assess the vascularity of flaps by measuring temperature, colour, capillary refill and pin prick. Several adjunctive measures have been proposed to aid the surgeon in his/her quest. These include the use of Doppler based instruments, tissue pH monitoring, skin temperature probes, tissue oxygen tension, near infrared spectroscopy, photoplethysmography, micro dialysis, flap glucose assessment as well as the use of fluorescent dyes amongst many others.

We propose the use of Fluorescein dye to predict the viability of flaps. Fluorescein is hygroscopic orange red powder, which dissolves readily in water. When viewed under UV light, it assumes a yellow green colour.

The test shall consist of injecting Fluorescein intravenously at 15 to 30 mg/kg. The dye diffuses across the capillary fluid compartment within 10-15 minutes. If the vascularity of the flap is maintained, fluorescence shall be visible at the flap thereby confirming viability of the flap. Areas of fluorescence and areas of low/absent fluorescence shall be marked and followed post-surgery.

This method of assessment of flap viability is simple, cost effective and requires little special equipment. It is more objective than traditional methods of flap monitoring such as observing colour, turgor or pin prick examination. Not only does such a method provide adequacy of flap perfusion, but it also allows for an objective comparison with respect to traditional methods of assessment of flap viability.

6.2 REVIEW OF LITERATURE

Despite rapid development in the field of reconstructive surgery, one core issue that persists to this day is the inability to accurately predict the viability of a flap.

Although many attempts have been made to elucidate elaborate as well as simple adjuncts to aid the surgeon in his decision making, the ideal technique remains to be found. Most techniques remain imperfect or expensive.[1]

Creech and Miller proposed that the ideal technique for assessment of flap viability should fulfill the following tenets:[2]

1) Simple and harmless to the patient
2) Rapid, repeatable, reliable, recordable and rapidly responsive
3) Accurate and inexpensive
4) Objective and applicable to all kind of flaps
5) Equipped with a simple display that could alert relatively inexperienced personnel to the development of circulatory impairment.

The most common technique used to check for flap viability is "Clinical examination". These may include all or a combination of the following:[3]

1) Colour
2) Turgor
3) Temperature
4) Capillary refill time
5) Bleeding following pin prick.

Although these methods are cheap and repeatable, they have certain limitations. The appreciation of colour, for example, is dependent on ambient lighting conditions.[4] The colour of the donor site may be different from the

recipient site as well. Skin turgor by itself has little proven diagnostic value and is dependent of the volume status of the patient.[5] Surface temperature is dependent on many factors including blood flow and changing metabolic rate of the underlying muscle.[6] Capillary refill time too has a wide variation amongst its users due to little standardization.[7] Hence, these techniques have to be supplanted by other more objective and reliable methods.

Fluorescein is a nontoxic dye which diffuses into the extracellular space.[8] When exposed to ultraviolet light, it gives off a yellow green fluorescence at a wavelength of 510 to 600 nm. This fluorescence persists in the extracellular space. In living tissue, the dye appears as bright yellow while areas which have no perfusion appear as dark blue.[9]

Fluorescein was initially used by Ehrlich in 1882, and then introduced to clinical use in 1931.[8] Skin fluorometry after an intravenous injection was studied by Lange and Boyd, who used the method to evaluate microcirculation of the skin.[10] Dingwall and Lord used the dye to predict the viability of tubed flaps in 1943.[3]

In 1973, Thorvaldsson and Grabb, studied 19 patients who required skin flaps with Fluorescein dye. In their study they discovered that 5 patients who had non-fluorescing areas after administration of Fluorescein necrosed post operatively.[11]

In 1977, McCraw et al. suggested that despite its along established record of use in various clinical scenarios, Fluorescein remains underused by reconstructive surgeons. In their study on 159 axial skin flaps and 126 myocutaneous flaps, they recommended routine fluorescein use whenever a major flap was manipulated.[12]

In 1979, Dibbell et al. developed a Fluorescein based quantitative method for predicting viability of skin flaps. In their study they noted that a few areas of non-fluorescence also survived, suggesting that these areas survived as a graft rather than a flap.[13]

Myers at al. in 1985, compared eight methods to predict the viability of skin flaps using Fluorescein. Both low dose and high dose experiments were carried out using a fluorometer, camera with filters, and the traditional Wood's lamp. The authors discovered that the traditional Wood's lamp is just as accurate as any other method.[14]

Pang et al., in 1986 suggested that a visual Fluorescein test performed 18 hours after flap surgery provided an accurate prediction of actual skin viability in flap surgery in pigs.[15]

Denneny et al., in 1986, published an experimental study on rats using Fluorescein elimination to determine perfusion of island flaps. They concluded that use of Fluorescein offers an advantage in assessment of an island flap.[16]

Galla et al, in 1992, performed a comparative analysis of tissue fluorescence as related to capillary perfusion in random pattern flaps, and concluded that skin flap fluorescence can be used for assessment of nutritive blood flow, viability of skin tissue and tissue survival in these flaps.[17]

Issing et al. in 1996, performed an experimental study in rats to compare pedicled skin flap viability by pH, temperature and fluorescein. They concluded that the method which gave the most exact prediction about viability was the fluorescein staining of the flaps.[18]

Bhattacharya et al. in 2006, conducted an experimental as well as clinical study examining the fluorescein technique for per operative evaluation of

vascularity. They suggested that the method was an easy, safe and reliable method to determine vascularity.[9]

In 2008, Losken et al. performed a study on fifty consecutive peri areolar mastectomy flaps, which were evaluated for skin flap viability using Fluorescein dye. They concluded that the test is sensitive, but underpredicts viability. They suggested that any area of non-fluorescence >4 cm^2 typically does not survive.[19]

In 2015, Beckler et al. performed a retrospective analysis for comparing skin paddle perfusion in those undergoing oromandibular reconstruction with fibular flap using clinical findings, indocyanine green and fluorescein dye. They suggested that the use of an intra operative dye may reduce the risk of partial necrosis in these patients.[20]

6.3 AIMS AND OBJECTIVES OF THE STUDY

1. To validate/negate the use of Fluorescein dye for flap viability in Plastic surgery.
2. To compare the use of Fluorescein dye to clinical examination of flap for viability.

7.0 MATERIALS AND METHODS

7.1 SOURCE OF DATA

A prospective study shall be conducted on 30 patients operated for various defects requiring flap coverage by the Department of Plastic surgery, Dayanand medical college and hospital, Ludhiana. Those part of the study shall have to satisfy the inclusion criteria and none of the exclusion criteria. The enrolment of the patients shall begin from April 1, 2019.

7.2 METHOD OF COLLECTION OF DATA

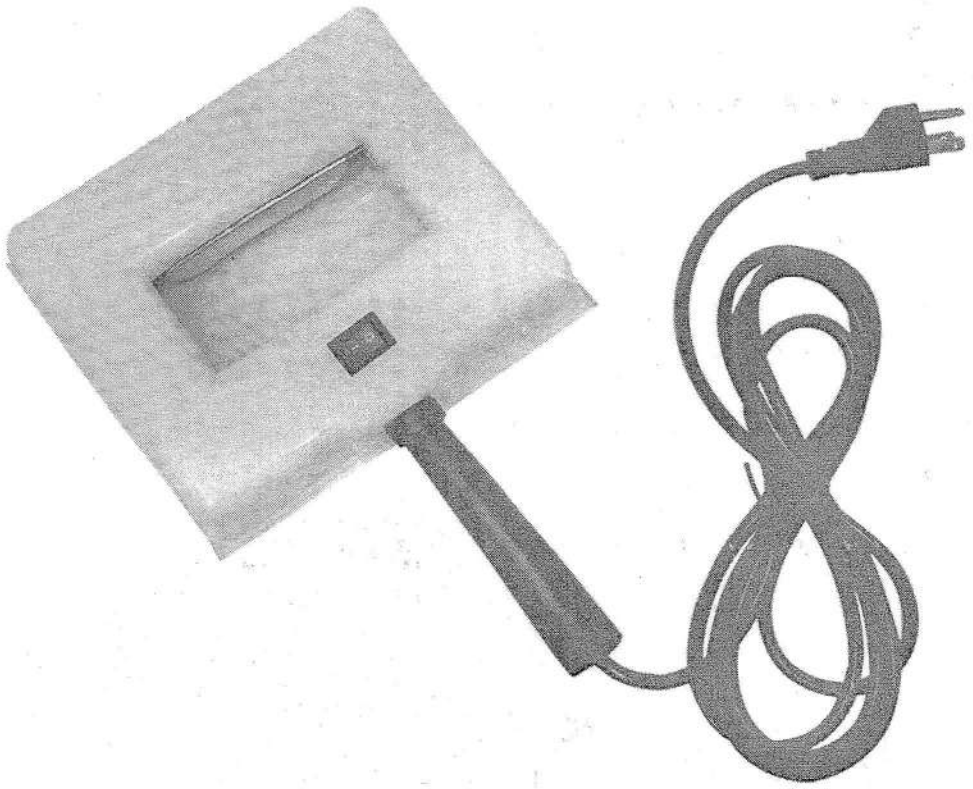
INCLUSION CRITERIA

The study shall be conducted on all consenting patients who undergo any type of flap surgery.

EXCLUSION CRITERIA

1. Patients who fail mandatory intradermal hypersensitivity test pre-operatively.
2. Patients who have a history of hypersensitivity to Fluorescein.
3. Patients who are breast feeding.

Pre operatively, the patient shall undergo an intradermal test dose of 0.05 mL. This shall be evaluated 30 minutes after injection to check for hypersensitivity. For free flaps, the dye shall be injected after completion of the arterio-venous anastomosis. The flap shall then be examined under ultraviolet light using a Wood's lamp. For all other flaps, the dye shall be injected intravenously after dissecting the flaps. After a few minutes, the operating room lights will be turned off and the flap shall be examined under ultraviolet light using a Wood's lamp (as pictured below). The cutaneous flap component (if any) will be de-epthelialized for improved visibility of the dye under ultraviolet light.



Wood's lamp for observing fluorescence under ultraviolet light

Areas of fluorescence and/or impaired fluorescence (if any) shall be examined, marked as well as documented onto a graph paper detailing the dimensions of the flap area.

Clinical examination shall include colour, capillary refill and needle prick examination at the time of surgery. The areas of adequate viability and/or non-viability shall be marked and documented onto a graph paper detailing the dimensions of the flap.

Outcome analysis shall be performed to compare intraoperative clinical assessment of flap viability versus fluorescein dye guided assessment and its comparison to the final outcome of the flap as assessed on post-operative day

5. Photo documentation shall be performed, and the areas of necrosis or non-viability shall be documented onto a graph paper detailing the areas of viable tissue and compared with operative assessment.

STATISTICAL ANALYSIS

Data will be described in terms of range; mean ± standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

Yes.

Intravenous fluorescein shall be given to patients who are part of the study. The dye has been widely used in other clinical branches especially Ophthalmology. It has a long safety record and is readily available.

7.4 HAS ETHICAL CLEARENCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached Herewith

LIST OF REFERENCES:

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17. Galla TJ, Anton-Lamprecht I, Kieser M, Saetzler RK, Messmer K. Comparative analysis of tissue fluorescence as related to capillary

perfusion in random pattern skin flaps. British journal of plastic surgery. 1992;45:578-85.

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PROFORMA

Name: Age: Sex:

Body weight:

MRD No.:

Date of Surgery:

Diagnosis:

Type of flap:

Anatomical location:

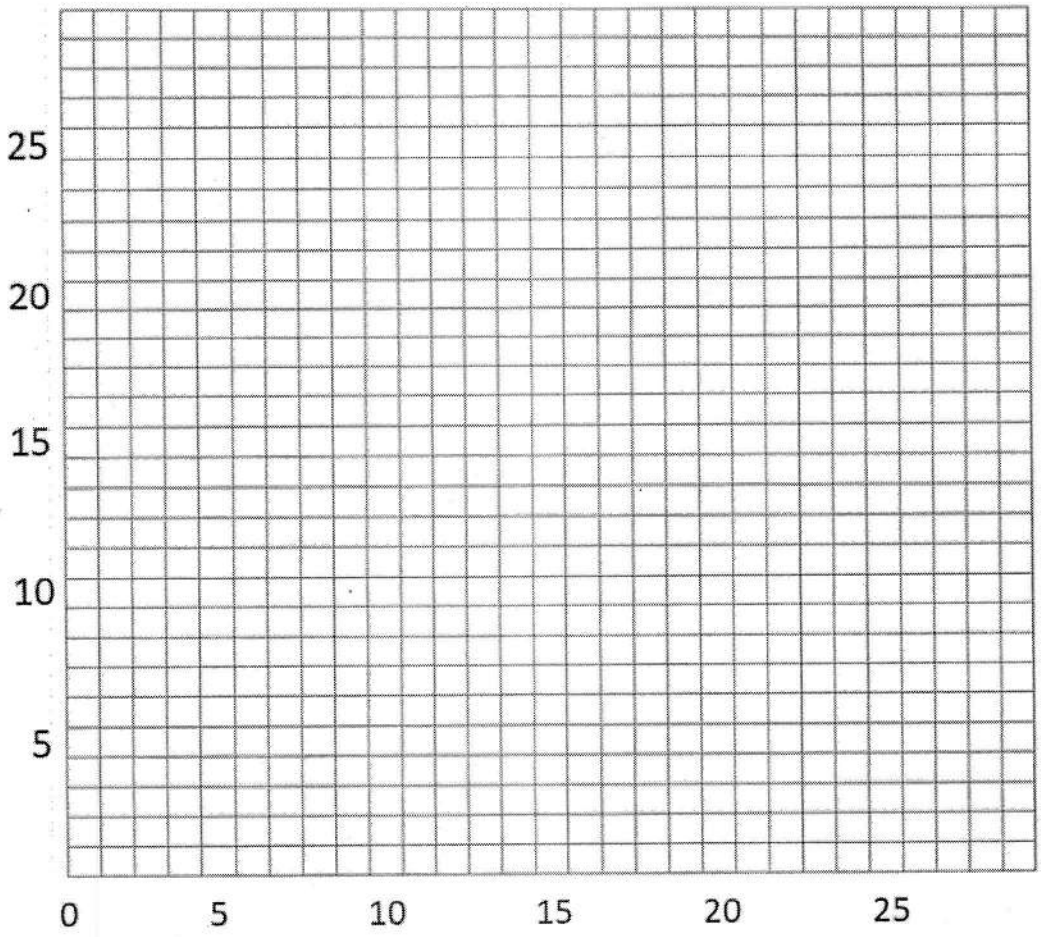
Signature of Candidate **Signature of Supervisor**

Operative assessment

A) Clinically viable area (as shaded in colour):

Colour	White	Pink	Purple	Black
Capillary refill	No Blanching	<3 seconds	3 seconds	>3 seconds	No refill
Needle prick	Arterial	Venous	Equivocal	Absent

Area in cm^2:



Scale: 1 box=$1cm^2$

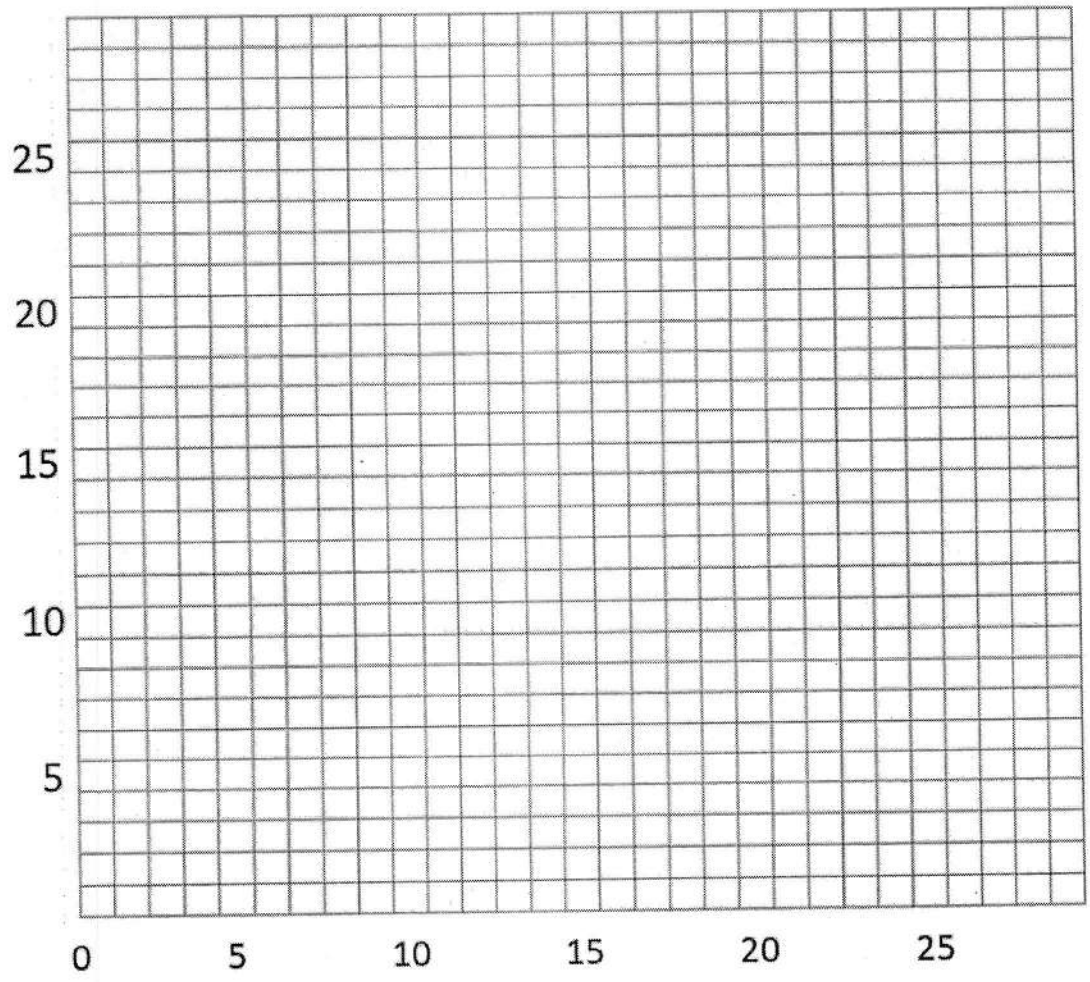
Signature of Candidate **Signature of Supervisor**

B) Areas of fluorescence in cm^2 (shaded in green):

Test dose given	Yes	No

Dose of Fluorescein given :

Type of Fluorescence (Area in cm^2)	Bright Fluorescence (Oblique lines)	Mottled (Dots)	No Fluorescence (Crosses)



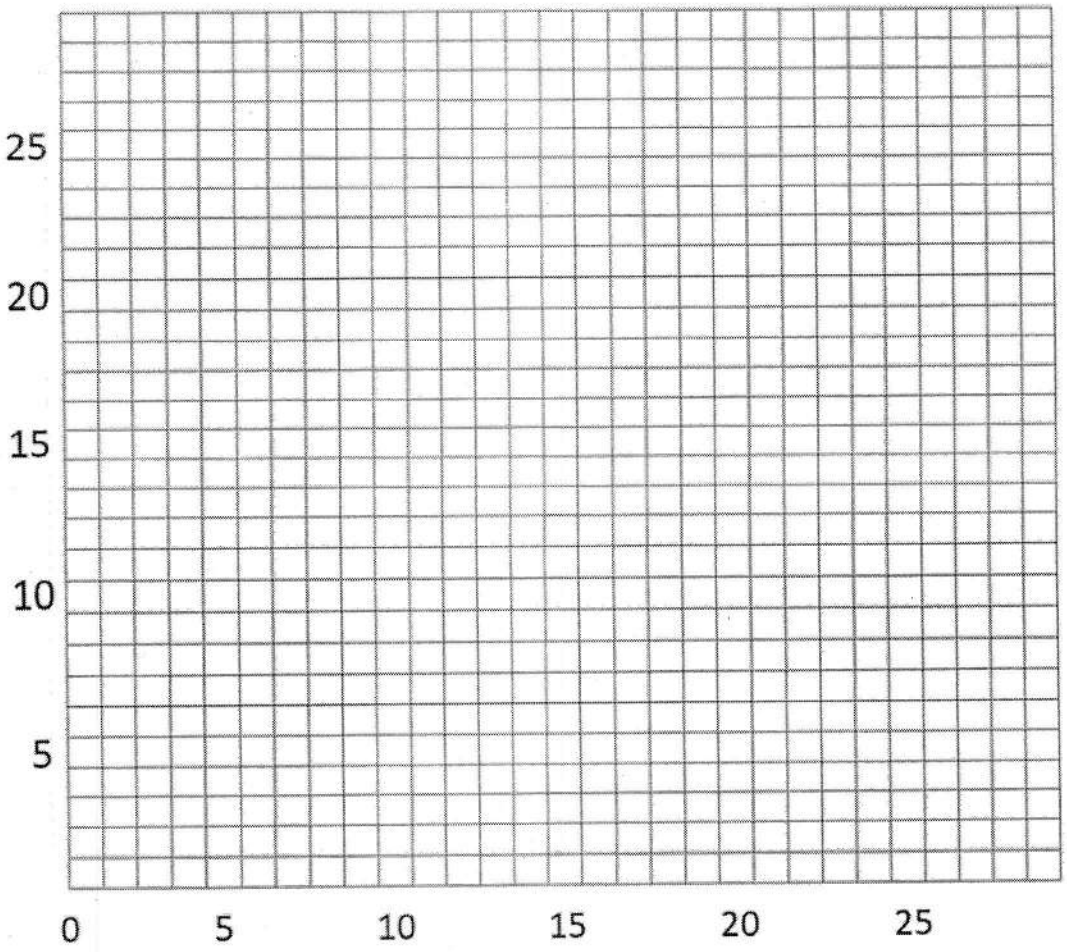
Scale: 1 box=1cm^2

Comments:

Signature of Candidate **Signature of Supervisor**

Post-operative assessment at day 5 after surgery

A) Clinically viable area (as shaded)
Area (cm^2):



Scale: 1 box=1cm^2

Signature of Candidate **Signature of Supervisor**

Comparison with Fluorescein assessment:

Complications:

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o.....

.....am willing to participate in the study **"USE OF FLUORESCEIN DYE FOR PERIOPERATIVE FLAP VIABILITY"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....	.....
Signature of the Witness	Signature of the patient
Name:	

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"USE OF FLUORESCEIN DYE FOR PERIOPERATIVE FLAP VIABILITY"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

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गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

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अन्वेषक के हस्ताक्षर

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विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"USE OF FLUORESCEIN DYE FOR PERIOPERATIVE FLAP VIABILITY" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____

ਪਤਾ _____

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ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____

ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ